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**POPULATION PHARMACOKINETICS AND  
PHARMACOKINETIC-PHARMACODYNAMIC  
MODELING OF ANTITUBERCULAR DRUGS**

By

**EMMANUEL CHIGUTSA**

Division of Clinical Pharmacology, Department of Medicine

UNIVERSITY OF CAPE TOWN

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Main Supervisor – A/Prof Helen McIlleron

Co-supervisor – Prof Carl Kirkpatrick

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*“Everything that is simple is false, and everything that is complex is unusable”*

*Paul Valérie*

University of Cape Town

## Contributions to the field

This thesis includes, but is not limited to the following contributions to the field:

### Full length original articles:

1. Emmanuel Chigutsa, Jonathan Monteleone, Daniel Weiner, Geraint Davies, Helen McIlleron. Clinical Trial Simulation to Optimize Study Design for Evaluating the Efficacy of Antitubercular Drugs. *Submitted*
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5. Emmanuel Chigutsa, Jan-Stefan Van Der Walt, Paolo Denti, Peter Smith, Helen McIlleron. A mixture model showing bimodal rifampicin absorption and clearance: Do drug transporter polymorphisms play a role? *5th International Conference on Pharmaceutical and Pharmacological Sciences*. Potchefstroom, South Africa. September 2009.

## ABSTRACT

### **Population Pharmacokinetics and Pharmacokinetic-Pharmacodynamic Modeling of Antitubercular Drugs**

Emmanuel Chigutsa – 2013

The pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol in 78 patients with tuberculosis were described using non-linear mixed effects modeling. Pharmacodynamic data was comprised of weekly sputum liquid culture (using mycobacterial growth indicator tubes) time to detection results from 144 patients during the first 2 months of treatment. The effect of drug exposure on patient outcomes was investigated. To determine the adequacy of ofloxacin drug exposure, the probability of attaining the required area-under-the-curve to minimum inhibitory concentration ratio (AUC/MIC) of ofloxacin was determined in 65 patients on treatment for multidrug resistant tuberculosis. To improve efficiency in the clinical development of new drug regimens, clinical trial simulation was used to determine the optimal study design for a study investigating the efficacy of a new antitubercular drug regimen.

The *SLCO1B1 rs4149032* polymorphism existed at a high frequency of 0.70 in South Africans and resulted in a 28% decrease in bioavailability of rifampicin. The rifampicin peak concentration was a significant predictor of the 2 month treatment outcomes. A semi-mechanistic time to event model was developed to analyze days to positivity (time to detection) data. The model was comprised of a biexponential decay model describing bacillary decline in sputum from patients, followed by a logistic model with a lag time for growth of the mycobacteria in liquid culture.

For the current 800 mg daily dose of ofloxacin, the probability of attaining an AUC/MIC target ratio of at least 100 was only 0.45.

Based on clinical trial simulation, the optimum parallel study design was comprised of 125 study participants in each of 2 arms to achieve a study power of at least 80%. Increasing the study length beyond 42 days reduced study power perhaps due to increased amounts of censored data.

Higher doses of rifampicin are required in the majority of South African patients with tuberculosis. A novel pharmacodynamic model of tuberculosis treatment is presented, which can be used for investigation of covariates such as drug exposure. Ofloxacin should be replaced with a more potent fluoroquinolone for treatment of multidrug resistant tuberculosis. Clinical trials should not be unduly long otherwise this may compromise study power.

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## LIST OF ABBREVIATIONS

AADAC – human arylacetamide deacetylase

AUC – area under the curve

AUC<sub>0-24</sub> - area under the curve from 0-24 hours

BMI – Body Mass Index

BSV – Between Subject Variability

CAR – constitutive androstane receptor

CART – Classification And Regression Tree Analysis

CDC – United States Centers for Disease Control and Prevention

CFU – colony forming unit

CI – confidence interval

CL – Clearance

C<sub>max</sub> – peak plasma concentration

CrCl – creatinine clearance

CRRS – Chest Radiograph Reading and Recording System

DNA – deoxyribonucleic acid

DOTS – Directly Observed Treatment Strategy

EBA – Early Bactericidal Activity

F – oral bioavailability

fAUC – unbound fraction of the area-under-the-curve

fAUC/MIC – ratio of the unbound fraction of the area-under-the-curve to the minimum inhibitory concentration

FDA – (United States of America) Food and Drug Administration

FOCE – First Order Conditional Estimation

h - hour

HIV – human immunodeficiency virus

KA – First order absorption rate constant

kg - kilogram

L - Litre

LBW – Lean Body Weight

LD – Linkage Disequilibrium

LJ – Lowenstein-Jensen

LLOQ – lower limit of quantification

m - metre

mg - milligram

MGIT – mycobacterial growth indicator tube

MIC – minimum inhibitory concentrations

min - minute

MTT – mean transit time

*M. tuberculosis* – *Mycobacterium tuberculosis*

MDR – multidrug resistant

μM – micromolar

NCA – non-compartmental analysis

NHLS – National Health Laboratory Service

NLME – non-linear mixed effects modeling

OATP-C – organic anion transporting polypeptide 1B1

OFV – Objective Function Value

P – probability of rejecting the null hypothesis when it is true (false positive result)

pdf – probability density function



P-gp – p-glycoprotein

PK-PD – Pharmacokinetic-Pharmacodynamic

PPV – Population Parameter Variability

PsN – Perl Peaks NONMEM

PTA – Probability of Target Attainment

Q – intercompartmental clearance

RCT - Randomised Controlled Trial

RNA – ribonucleic acid

ROC – Receiver Operating Characteristic

RSE – Relative Standard Error

SLCO1B1 – Solute Carrier Organic Anion Transporter Family 1B1

SNP – Single Nucleotide Polymorphism

TB – tuberculosis

TBTC – TB Trials Consortium

Tmax – time to peak plasma concentration

TNTC – too numerous to count

TTD – time to detection

V – Apparent volume of distribution

VPC – Visual Predictive Check

WHO – World Health Organization

WSV – Within Subject Variability

y - years

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# **1. INTRODUCTION and LITERATURE REVIEW**

## **1.1 Tuberculosis – The disease, the treatment, the past and the present**

Tuberculosis (TB) is an extremely old disease caused by the tubercle bacillus which was discovered by Robert Koch in 1882 (Sakula 1982). *Mycobacterium tuberculosis* (*M. tuberculosis*) is the causative agent of TB and genetic analysis has shown this bacterium to have been in existence as many as 3 million years ago (Gutierrez *et al.* 2005). Thus humankind and *M. Tuberculosis* have been in co-existence and co-evolution for a very long time, both species originating in East Africa (Gutierrez *et al.* 2005) and thereafter migrating from Africa to the rest of the world. Molecular identification of DNA of *M. Tuberculosis* in skeletal remains discovered in tombs of ancient Egyptians shows that the disease was present in this population before 3000 BC (Haas *et al.* 2000). Further molecular evidence of *M. Tuberculosis* infection in a Middle Eastern population dates back more than 9000 years ago (Hershkovitz *et al.* 2008). The disease is responsible for many deaths of mankind throughout the ages.

Globally today, about 8.7 million new infections occur annually with 1.4 million deaths, making TB the second leading cause of death from an infectious disease worldwide, after human immunodeficiency virus (HIV) infection according to the World Health Organization (WHO 2012). While these figures are alarmingly high, they represent somewhat slow but positive developments since the mortality rate has decreased by 41% since 1990 and the number of new cases decreased by 2.2% between 2010 and 2011 (WHO 2012).

Although more than two-thirds of the estimated 1.4 million deaths globally occurred in HIV negative individuals (WHO 2012), HIV infection remains an important contributor to TB

incidence and mortality in sub-Saharan Africa. About one-third of new TB infections in sub-Saharan Africa (and 50% percent for South Africa) are attributable to HIV infection, yet this figure is less than 5% for the rest of the world (Corbett *et al.* 2003). This high frequency of HIV-TB coinfection means that drug-drug interactions in patients receiving treatment for the 2 diseases are important, especially between rifamycins and HIV protease inhibitors (Zhang *et al.* 2012; Zhang *et al.* 2012). HIV-TB coinfection also means a very high pill burden for patients since both diseases are treated with multi-drug regimens. Coupled with treatment for other opportunistic infections, patients can easily be required to swallow more than 10 tablets/capsules in the morning.

Historically in developed countries, cases of TB increased due to overcrowding as a result of industrialization, then started to decrease from the middle to late 19<sup>th</sup> century continuing into in the 20<sup>th</sup> century due to improved sanitation, housing and living conditions (Philip 1928), even before any drug treatment for TB was used. The halving of the death rate due to TB infection in New York over a 20 year period (Anonymous 1921) in the absence of pharmacological intervention was similarly attributed to improved working and socio-economic conditions (Weber 1948), an improved public health care system of detecting, reporting and registering TB cases, and even segregation of advanced TB cases (Anonymous 1921).

In spite of the steady downward trend in the number TB deaths noted above, it cannot be disputed that the discovery of streptomycin in 1944 (Schatz *et al.* 1944) was a key development towards treatment of a potentially fatal disease, although the number of infections was already on the decline. Investigation of the drug in TB patients in a study whereby patients were randomized to either bed rest alone or bed rest plus streptomycin and

observed for 6 months before comparing the number of deaths in each arm (British Medical Research Council 1948) is arguably the first ever recorded controlled clinical trial (DeMets and Califf 2011). It was not long after introduction of streptomycin that mycobacterial resistance to the drug was observed, with one study reporting strains collected from 11 of 13 patients during treatment being at least 32 times less sensitive to streptomycin than those collected before initiation of treatment (Crofton and Mitchison 1948).

Streptomycin treatment involves inconvenient parenteral administration while most antitubercular drugs are administered via the oral route. An important oral drug was isoniazid which came to the fore in 1952 (Gomez 1952; Mackaness and Smith 1952). Soon after the introduction of isoniazid therapy, development of resistance was reported (Coletso 1953; Selkon *et al.* 1964). Probably the most important drug class in TB pharmacotherapy right up to this day are the rifamycins, which were first isolated in 1957 (Sensi 1983) and subsequently used for treatment of TB in the early 1960s (Lucchesi *et al.* 1962; Zorzoli *et al.* 1962). A common member of this drug class is rifampicin, discovered in an Italian laboratory around 1966 (Maggi *et al.* 1966). Once again, resistance to rifampicin was soon reported (Stottmeier 1976). Pyrazinamide is also an important drug in TB treatment nowadays, even though it was first used for TB in 1952 and rapid development of resistance was noted amongst patients being treated for TB around the same time (Yeager *et al.* 1952). Pyrazinamide was subsequently tested in combination with isoniazid in 1954 (Campagna *et al.* 1954). Ethambutol is a bacteriostatic antitubercular drug first reported to be investigated in 1962 (Delaude *et al.* 1962) and later was used in combination with rifampicin (Gyselen *et al.* 1968; Jeanes *et al.* 1972). The 4 oral drugs, rifampicin, isoniazid, pyrazinamide and ethambutol now form the composition of most first-line regimens in many parts of the world (WHO 2012), and in South Africa as well (Health and Medical Publishing Group 2010). The



regimen typically consists of all 4 drugs for 2 months (intensive phase) followed by rifampicin and isoniazid for 4 months (continuation phase).

After a drought in research towards developing new antitubercular drugs, a few new drugs are being developed and are in clinical trials e.g. bedaquiline, formerly known as TMC207 (Diacon *et al.* 2009), PA-824 (Ginsberg *et al.* 2009) and more recently delamanid (Gler *et al.* 2012). Some of these drugs are at very advanced stages in the drug development pipeline, with the manufacturers of bedaquiline (Janssen Research and Development) and delamanid (Otsuka Pharmaceuticals) recently filing new drug applications with the United States Food and Drug Administration (FDA) and European regulatory agencies respectively (Willyard 2012). Most of these new drugs have long elimination half lives and possess activity in cases of TB resistant to other antitubercular drugs. However, it may be a long time before most new drugs are developed to be part of optimal regimens thereafter making it to the mainstream African public health system in an affordable manner. Until then, options for treatment of TB remain limited to the few drugs available which have been in use for more than 6 decades.

### **1.1.1 Phenotypic Subpopulations of *M. Tuberculosis***

*M. Tuberculosis* is a very slowly replicating bacterium with an *in vitro* doubling time of about 24 hours in Middlebrook 7H9 liquid media (Straus and Wu 1980; Ginsberg and Spigelman 2007), but varying from 18-54 hours under similar growth conditions (Gill *et al.* 2009). Most other bacteria replicate much more rapidly in culture, e.g. *Staphylococcus aureus* which has a doubling time of about 24 minutes (Domingue *et al.* 1996). In patients with TB, at least 2 different subpopulations have been described in terms of metabolic activity and replication rate: those that are actively replicating and are killed rapidly by antitubercular drugs (Jindani *et al.* 2003), and those that are non-replicating which can lie dormant in the human host for

very long periods of time and are less sensitive to bactericidal activity of drugs (Wayne and Hayes 1996). The dormant bacteria can result in latent infection for several decades before clinical disease appears (latency) due to decreased immune surveillance or other unknown factors (Lillebaek *et al.* 2002). As will be discussed later, different drugs can have different effects on these subpopulations (Paramasivan *et al.* 2005) and these differences can be quite pronounced whereby some drugs act only on actively replicating bacilli whilst others may act only on dormant bacteria whilst other drugs may kill both subpopulations (Jindani *et al.* 1980). The dormant mycobacteria are killed much more slowly and are generally less susceptible to antibiotics than the actively dividing bacteria.

The terms latency, dormancy and persistence are often used interchangeably and may be confusing, hence will be defined here according to a summary by Gomez and McKinney (2004). Latency describes the state of a tuberculous lesion that does not produce clinical symptoms which is induced by an initial vigorous immune response to contain the infection. Dormancy relates to the bacteria adopting mechanisms to survive under stressful conditions of low oxygen tension, acidic conditions and amino acid starvation in parts of the lung and they undergo phenotypic changes whereby they do not replicate (Gomez and McKinney 2004). These phenotypic changes also make the bacteria more tolerant to antibiotics (Grant *et al.* 2012). Persistence refers to the ability of the mycobacteria to 'persist' in the face of stressful conditions including drug pressure (Gomez and McKinney 2004). The slowly killed bacteria that remain after the initial killing of actively dividing organisms are frequently called 'persisters' which appear to be genetically identical to drug sensitive organisms but are not replicating (Zhang *et al.* 2012).

### 1.1.2 Drug resistance in tuberculosis

Resistance to antimycobacterial drugs is a phenomenon that has been present almost as long as the treatments have been in existence and has been shown to quickly appear with monotherapy. It has been found that development of resistance mutations takes place spontaneously and at random (David 1970) with the mutations naturally occurring in strains not previously exposed to drugs (Tsukamura 1972). *M. tuberculosis* seems to have a lower mutation rate for rifampicin compared to isoniazid, with ethambutol having a higher mutation rate than the other 2 drugs (David 1970).

Multidrug resistant tuberculosis (MDR-TB), which is defined as TB resistant to rifampicin and isoniazid is of increasing concern. Globally, among previously treated TB patients, 18.5% have MDR-TB (Zignol *et al.* 2006) and 3.7% of all new TB cases are MDR-TB (WHO 2012). The treatment of MDR-TB is very long (at least 18-24 months) and is characterized by high rates of drug toxicity particularly ototoxicity of aminoglycosides (especially kanamycin) (Voogt and Schoeman 1996; de Jager and van Altena 2002) and neurological effects of cycloserine and terizidone (Shmelev *et al.* 1975). The fluoroquinolones are relatively well tolerated at currently recommended doses but have been associated with tendonitis and dysglycaemias (Janssen-Ortho 2006) as well as cardiac arrhythmias via QT prolongation (Falagas *et al.* 2007).

MDR-TB treatment success rates are about 62% (95% credible interval of 58-67%) according to a meta-analysis of 34 clinical studies (Orenstein *et al.* 2009). Although these success rates are comparable with the global average of 76% observed in countries implementing the WHO TB detection and treatment strategies (Raviglione *et al.* 1997), other studies have shown that MDR-TB or monoresistance to rifampicin or isoniazid is associated with

significantly higher failure rates (Espinal *et al.* 2000). The quinolones are a very important drug class in the management of MDR-TB and have improved cure rates although development of resistance to quinolones is a concern (Ginsburg *et al.* 2003; Ginsburg *et al.* 2003; Migliori *et al.* 2008). In patients treated for MDR-TB, the median sputum culture conversion times are reported to be 2-4 months (Yew *et al.* 2000; Holtz *et al.* 2006; Prasad *et al.* 2006). However a study in the Western Cape found that between 2001 and 2002, 45% of MDR-TB patients had sputum culture conversion in a median (range) time of 5 (2.4 – 45) months (Shean *et al.* 2008). Thus cure rates appear to be lower in South Africa, coupled with longer times to sputum conversion when compared with European studies. Other patients failed to respond to treatment despite drug susceptibility tests showing susceptibility to their second-line drug regimens and receiving observed drug doses as inpatients (Shean *et al.* 2008). Their failure to respond raises questions about the adequacy of systemic drug concentrations in relation to local drug susceptibility profiles.

## **1.2 Pharmacology of antitubercular drugs**

### **1.2.1 Rifampicin**

The antimycobacterial activity of rifampicin is based on its binding to and inhibition of bacterial deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase, the enzyme responsible for DNA transcription (Wehrli 1983) thus ultimately interfering with synthesis of proteins necessary for survival of the bacterium. It then stands to reason that if a mutation occurs in the gene encoding RNA polymerase subunit beta (*rpoB*), this may result in subsequent amino acid changes which alters the conformation of the protein and leads to defective binding of rifampicin thus rendering the mycobacterium insusceptible to rifampicin

(Telenti *et al.* 1993). *rpoB* mutations have subsequently been shown to be the culprit in the majority of clinical *M. tuberculosis* strains resistant to rifampicin (Suzuki *et al.* 1995; Ahmad *et al.* 2000).

#### **1.2.1.1 Rifampicin pharmacokinetics**

Rifampicin pharmacokinetics is known to be highly variable (Wilkins *et al.* 2008) with more than 10 fold interindividual differences in peak plasma concentrations (C<sub>max</sub>) and half lives (Boman 1974). The drug has a bioavailability of about 68% during chronic administration compared to 93% for single dose administration (Loos *et al.* 1985), which indicates autoinduction of first-pass effect (Smythe *et al.* 2012). Rifampicin is about 80% protein bound, mainly to albumin, and it distributes well throughout the body and body fluids (Acocella 1978). Rifampicin is reported to have a rather short half life of about 2.5 hours following a single 600 mg oral dose, undergoing dose dependent pharmacokinetics i.e. saturable elimination at doses higher than 450 mg (Acocella 1978) due to saturation of the biliary transport mechanisms (Acocella 1983).

About half of the rifampicin dose is eliminated unchanged through biliary excretion ultimately appearing in the faeces, whilst the other half is found in the urine as a combination of unchanged drug (10%) and metabolites (Sanofi-Aventis 2007). The drug has a volume of distribution of about 55 L and undergoes enterohepatic recycling (Sandoz 2008). Food has been reported to decrease the rate of absorption of rifampicin as evidenced by an increase in the time to peak plasma concentration, T<sub>max</sub> (Zent and Smith 1995) coupled with a decrease in the magnitude of the C<sub>max</sub> (Jeanes *et al.* 1972). Food has also been shown to decrease the bioavailability of rifampicin (Zent and Smith 1995) although another study found no effect of food on overall drug exposure despite showing reduced C<sub>max</sub> (from a mean of 10.5 mg/L for

fasted patients to a mean of 7.5 mg/L with a meal) and increased T<sub>max</sub> (Peloquin *et al.* 1999). The absorption of rifampicin is reported to be quite variable (Wilkins *et al.* 2008) with subgroups of 'smooth absorbers' (short absorption lag phase) and 'low absorbers' (long absorption lag phase) having been described (Peloquin *et al.* 1997). The expected average area-under-the-concentration-time-curve over 24 hours (AUC<sub>0-24</sub>) following standard doses ranges from 25-80 mg.h/L (Peloquin *et al.* 1999; McIlleron *et al.* 2006; Wilkins *et al.* 2008). Lower plasma rifampicin concentrations have been reported in HIV positive patients when compared with HIV negative patients (McIlleron *et al.* 2006) although the findings barely reached statistical significance. More recently, it has been shown that administration of antiretroviral drugs in HIV positive patients had no significant effects on the pharmacokinetics of the first-line antitubercular drugs (McIlleron *et al.* 2012).

Rifampicin is a potent enzyme inducer and the induction of its own metabolism was noticed as early as 1972 (Jeanes *et al.* 1972). The major metabolite of rifampicin is 25-desacetyl rifampicin, although it was only in 1997 when the B-esterase family in human liver microsomes were shown to be the mediators of this biotransformation (Jamis-Dow *et al.* 1997). More recently, the exact enzyme responsible for deacetylation of rifampicin has been found to be human arylacetamide deacetylase, AADAC (Nakajima *et al.* 2011). Interestingly, Jeanes *et al.* reported a decrease in urinary recovery of the desacetyl metabolite as time on treatment increased and they attributed this to increased biliary excretion (Jeanes *et al.* 1972) thus pointing towards induction in biliary drug transporters in addition to enzyme induction. Rifampicin induces multiple phase I and phase II drug metabolising enzymes and drug transporters such as p-glycoprotein (P-gp) through binding to and subsequent activation of the pregnane X receptor (PXR) (Rae *et al.* 2001; Chen and Raymond 2006). Rifampicin has the greatest induction potential of the currently available rifamycins, followed by rifapentine

(Li *et al.* 1997). Although rifampicin has greater induction potential on a micromolar ( $\mu\text{M}$ ) scale, at clinically relevant doses it has recently been shown that rifapentine is a stronger inducer of CYP3A (Bliven-Sizemore *et al.* 2011). The strong induction properties of rifampicin give rise to several clinically important drug interactions often resulting in the necessity to adjust the dose of the affected drug (Zhang *et al.* 2012), or change the substrate drug, or to use rifabutin as an alternative rifamycin which appears to cause less enzyme induction.

### **1.2.1.2 Rifampicin disposition and drug transporters**

The drug transporter P-gp (coded for by the *ABCB1* gene) is an efflux protein distributed in specific areas of the body including the intestines, blood-brain-barrier and the liver (Thiebaut *et al.* 1987). Rifampicin is a well recognised substrate of P-gp (Schuetz *et al.* 1996). Rifampicin is also an inducer of P-gp expression and the extent to which this induction occurs varies significantly between individuals. Polymorphisms in the *ABCB1* gene, have been shown to influence the expression and activity of P-gp (Hoffmeyer *et al.* 2000; Sakaeda *et al.* 2001; Goto *et al.* 2002; Moriya *et al.* 2002; Nakamura *et al.* 2002). A study conducted specifically in patients with very low rifampicin concentrations found that administration of verapamil (a known P-gp inhibitor) increased the 4 hour rifampicin area under the curve ( $\text{AUC}_{0-4}$ ) by 84%. This group of patients was also found to have delayed drug absorption showing the effect of high P-gp activity (Prakash *et al.* 2003) in the gastrointestinal tract. It is therefore possible that polymorphisms of the *ABCB1* gene resulting in altered P-gp activity and/or expression may influence rifampicin pharmacokinetics.

Differences in frequencies of *ABCB1* polymorphisms have been observed between Caucasians and African Americans. Two synonymous single nucleotide polymorphisms

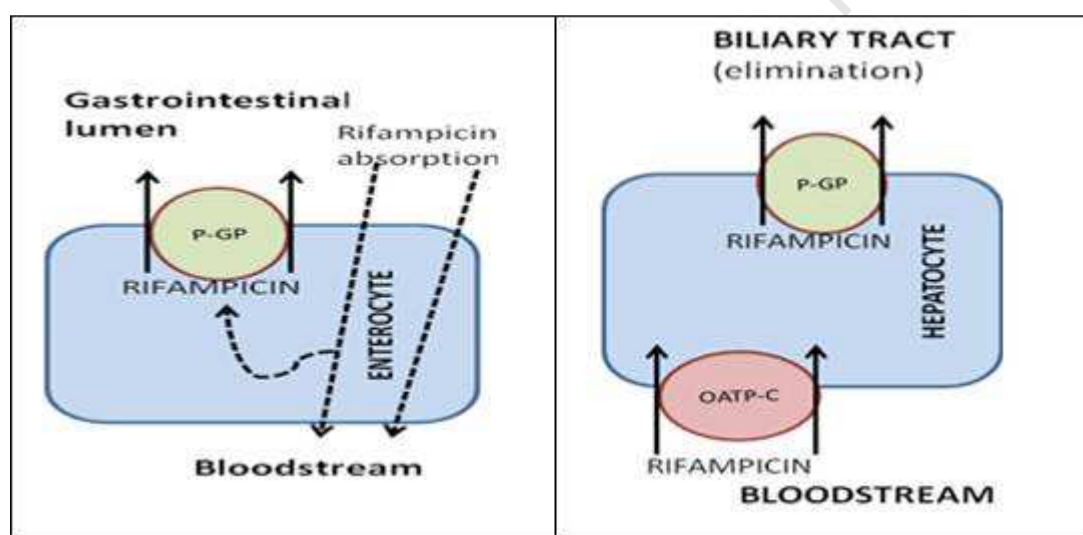
(SNPs) (*C1236T* and *C3435T*) and a nonsynonymous SNP (*G2677T*) were found to be linked and were collectively called *ABCB1*\*2. The *ABCB1*\*2 haplotype was found in 62% of European Americans and 13% of African Americans (Kim *et al.* 2001). In the same study, this haplotype resulted in 40% lower AUC of the P-gp probe drug fexofenadine, further demonstrating the clinical significance of P-gp polymorphism. The *C3435T* polymorphism occurs at an overall frequency of 39% in Japanese subjects (Sakaeda *et al.* 2001), showing differences with Caucasians (Cascorbi *et al.* 2001; Kim *et al.* 2001). The frequency in West Africans (Ghanaians) is reported to be 10% (Schaeffeler *et al.* 2001).

Hepatocellular uptake (from bloodstream into hepatocytes) of rifampicin is mediated by organic anion transporting polypeptide 1B1 (also known as OATP-C; coded for by the solute carrier organic anion transporter, *SLCO1B1* gene) and *SLCO1B3* (OATP-8) transporters (Kim 2003; Tirona *et al.* 2003) which are specifically located in the liver (Konig *et al.* 2000; König *et al.* 2000). The enterohepatic recycling of rifampicin is probably due to hepatocellular uptake mediated by OATP1B1, followed by efflux into the bile mediated via P-gp (Faber *et al.* 2003). Tirona *et al.* however showed that rifampicin is primarily transported by *SLCO1B1* and only to a modest extent by *SLCO1B3* (Tirona *et al.* 2003). There is also far more *SLCO1B1* than *SLCO1B3* in the liver (Abe *et al.* 2001), therefore *SLCO1B1* polymorphisms are probably more important. Various SNPs within *SLCO1B1* have been shown to result in very high plasma drug concentrations of several hydroxy-methyl-glutaryl coenzyme A reductase inhibitors (statins). This can be explained by the variant protein having impaired function, hence less hepatic uptake and subsequent biliary excretion of statins takes place (Niemi *et al.* 2004).



The frequencies of functional polymorphisms of *SLCO1B1* vary across populations. In one study, the most common SNP leading to decreased *SLCO1B1* activity in African Americans was *G1463C* (9% frequency), whilst the most common in Caucasians was *T521C* (14% frequency) (Tirona *et al.* 2001). The frequencies of *G1463C* in Caucasians was 0% and the frequency of *T521C* in African Americans was 2% (Tirona *et al.* 2001) and different frequencies have been reported in Japanese subjects (Nozawa *et al.* 2002). This shows that results are not comparable between different ethnic groups and each group requires separate investigation.

The diagram below illustrates the role of drug transporters in rifampicin disposition.



**Figure 1.1:** Diagram of rifampicin absorption in the gastrointestinal lumen and excretion in the bile (Chigutsa *et al.* 2009).

### 1.2.1.3 Pharmacogenetic determinants of rifampicin pharmacokinetics

Unlike the famous *NAT-2* polymorphism influencing isoniazid pharmacokinetics, there is scarce information related to polymorphisms of drug transporters or drug metabolizing enzymes potentially affecting rifampicin disposition. The implications of some *SLCO1B1* polymorphisms for rifampicin pharmacokinetics were explored by Weiner *et al.* The authors

found that the *SLCO1B1* C463A polymorphism decreased rifampicin exposure by 36% in a mixed cohort of Caucasians and Africans (Weiner *et al.* 2010). It is unclear whether highly variable rifampicin pharmacokinetics reported in several studies can possibly be explained by variation in transport proteins governing its absorption (P-gp) and elimination (*SLCO1B1* for hepatocellular uptake, and P-gp for biliary excretion). Apart from SNPs in the transporters in Figure 1.1 possibly having an impact on drug concentrations, polymorphisms of nuclear receptors PXR (NR1I2) and the constitutive androstane receptor (CAR) which regulate the expression of *ABCB1* transcription (Synold *et al.* 2001; Burk *et al.* 2005) can indirectly impact on drug concentrations. Decreased PXR and/or CAR activity decreases *ABCB1* expression and the converse is true (Synold *et al.* 2001; Burk *et al.* 2005). The C63396T polymorphism in the *PXR* gene is associated with subtherapeutic concentrations of the antiretroviral drug atazanavir (Siccardi *et al.* 2008). Various SNPs of PXR exist in different ethnic groups at different frequencies (Zhang *et al.* 2001; Wang *et al.* 2008). The impact of *PXR* polymorphisms on rifampicin pharmacokinetics needs evaluation in combination with *ABCB1* and *SLCO1B1* polymorphisms.

#### 1.2.1.4 Rifampicin pharmacodynamics

Rifampicin has both early bactericidal activity [EBA] (average rate of sputum bacillary decline in the first two days of therapy is 0.17 log<sub>10</sub> colony-forming units [CFU] per mL of sputum per day for a 10mg/kg dose (Jindani *et al.* 2003)) and sterilizing activity (ability to kill less metabolically active bacteria [persisters] throughout treatment as evidenced by a decline of 0.096 log<sub>10</sub> CFU per mL of sputum per day for a 10mg/kg dose from 2-14 days (Jindani *et al.* 2003)). Although its EBA is superseded by that of isoniazid which rapidly kills most of the actively dividing bacteria within 2 days of treatment (Jindani *et al.* 2003), a significant part of continued bacterial kill beyond 2 days of therapy can be attributed to

rifampicin, although the role of pyrazinamide will also become apparent and will be discussed in further detail later. Rifampicin's ability to kill bacilli that are dormant most of the time (but with occasional spurts of metabolism) (Dickinson and Mitchison 1981) renders it a very good sterilizing drug. The sterilizing activity of a drug is defined by its ability to kill these dormant bacilli or other slowly growing bacilli usually found in acidic environments provided by areas of necrosis (Blumberg *et al.* 2003). These types of bacteria can persist beyond the early months of treatment and are responsible for relapse of TB infection (Blumberg *et al.* 2003).

#### **1.2.1.5 Rifampicin pharmacokinetics-pharmacodynamics**

The AUC adjusted by the minimum inhibitory concentration (MIC) is the best known correlation with the bactericidal activity of rifampicin (Jayaram *et al.* 2003). The C<sub>max</sub> to MIC ratio also shows a good correlation with kill rates, however not as strong as that for the AUC, whilst the time above the MIC is rather weakly correlated (Jayaram *et al.* 2003). The C<sub>max</sub> has been shown to be an important determinant of the post-antibiotic effect of rifampicin, the duration of which lasts about 5 days *in vitro* and increases linearly with the C<sub>max</sub> (Gumbo *et al.* 2007). The C<sub>max</sub> is strongly correlated with emergence of resistance thus higher C<sub>max</sub>'s (target free C<sub>max</sub>/MIC ratio of  $\geq 175$ ) lead to lower levels of emergence of resistance, whilst the AUC remains important for rate of mycobacterial kill (Gumbo *et al.* 2007). The figure below highlights the fact that the mycobacterial kill of rifampicin is driven by the total exposure, since the level of killing was the same regardless of the dosing schedule. The study was conducted in an *in vitro* hollow fibre system aimed at mimicking pharmacokinetic-pharmacodynamic (PK-PD) systems *in vivo*.

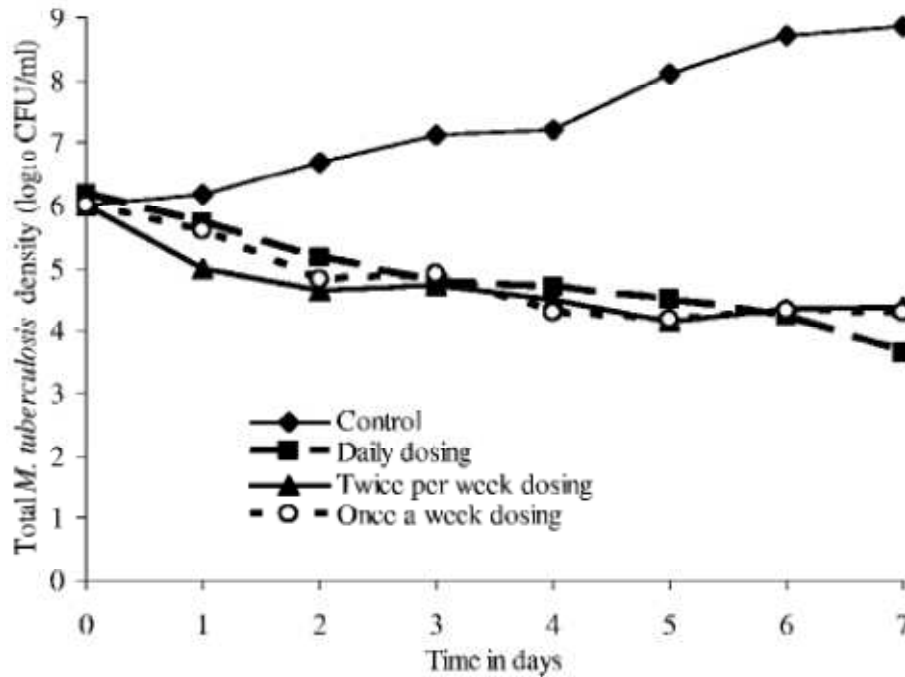


Figure 1.2: Response of *M. Tuberculosis* to rifampicin administered in 3 dose schedules. From Gumbo *et al*, 2007

The PK-PD studies above have been conducted either *in vitro* or in animals (mice) and unfortunately there is paucity in the literature with respect to rich exposure-response data from patients. The studies for exposure response have mainly been limited to early bactericidal studies lasting only a few days in duration (Jindani *et al.* 1980; Jindani *et al.* 2003; Diacon *et al.* 2007) which have limitations when trying to extrapolate to final patient outcomes after several months of treatment. One study though investigated the effect of rifabutin AUC on acquisition of rifamycin resistance or relapse of treatment and found these conditions to be associated with lower drug exposure (Weiner *et al.* 2005). It must be mentioned that this study only had 6 cases vs. 94 controls therefore such findings need confirmation.

### 1.2.2 Isoniazid

Isoniazid can be considered a prodrug in that it exerts its antimycobacterial action only after oxidation by mycobacterial catalase-peroxidase (katG). The activated isoniazid then binds to an enoyl-acyl carrier protein reductase (inhA) resulting in irreversible inactivation of inhA which is required for synthesis of essential fatty acids (mycolic acid) in mycobacteria (Johnsson *et al.* 1995; Lei *et al.* 2000).

#### 1.2.2.1 Isoniazid pharmacokinetics and N-acetyltransferase-2 polymorphism

Isoniazid (like rifampicin) is a drug reported to have highly variable pharmacokinetics (Wilkins *et al.* 2011). This variability has been partly attributed to polymorphism in the enzyme responsible for metabolising isoniazid, N-acetyltransferase 2 (NAT-2) (Evans and White 1964). Indeed, at a phenotypic level, after the introduction of isoniazid therapy for TB, it was not long before it was noted that some people excreted the drug metabolised to acetylisoniazid twice as fast as others (Bonicke and Reif 1953). It was shown that rapid acetylators acetylated isoniazid 5-6 times more rapidly than slow acetylators (Ellard and Gammon 1976). This phenomenon led to some researchers suggesting that knowledge of patient acetylator status could improve TB therapy (Mason and Russell 1971). The authors may not have realised it at that time, but drugs such as isoniazid slowly gave impetus to the general concept of personalized medicine, partly touted nowadays as pharmacogenetics although patients are not currently dosed according to NAT-2 genotype or phenotype.

Several polymorphisms in the NAT-2 gene lead to an enzyme with decreased catalytic activity (Fretland *et al.* 2001) which may result in toxic drug concentrations. Similar to several other pharmacogenetic studies conducted in diverse ethnic groups (Kita *et al.* 2001; Ait Moussa *et al.* 2002; Chen *et al.* 2006), studies in the Western Cape in South Africa have

also characterised the relationships between isoniazid exposure and *NAT-2* genotypes (Parkin *et al.* 1997; Schaaf *et al.* 2005; Donald *et al.* 2007; McIlleron *et al.* 2009) very well, even extending the classification of slow and fast acetylators to include intermediate acetylators. This trimodality in isoniazid acetylation has been shown to become apparent from as early as the first 3 months of life (Kiser *et al.* 2012). There is wide geographic variation in the distribution of *NAT-2* polymorphisms (Sabbagh *et al.* 2008). Some *NAT-2* alleles (e.g. *NAT2\*14*) are African specific and a high prevalence of slow and intermediate acetylators has been found in African populations due to the common *NAT2\*5*, *\*6* and *\*14* polymorphisms (Matimba *et al.* 2009). Polymorphisms of *NAT-2* and cytochrome P4502E1 (*CYP2E1*) have been linked to hepatotoxicity and peripheral neuropathy of isoniazid, although data are conflicting (Ohno *et al.* 2000; Huang *et al.* 2002; Huang *et al.* 2003; Vuilleumier *et al.* 2006; Cho *et al.* 2007; Possuelo *et al.* 2008) and there is no real consensus.

#### **1.2.2.2 Isoniazid absorption and disposition**

As can be deduced from the preceding paragraphs, isoniazid is a very well studied drug, together with the influence of *NAT-2* genetic polymorphism which has been repeatedly studied over decades. Pertaining to other determinants of its pharmacokinetics, the bioavailability of isoniazid is decreased by food (Zent and Smith 1995) and higher exposure may be achieved by administration on an empty stomach. Another study reported food to decrease the *C<sub>max</sub>* by half (from 5.5 mg/L down to 2.7 mg/L) and also nearly double the *T<sub>max</sub>* (Peloquin *et al.* 1999) although the authors did not find a statistically significant effect of food on overall drug exposure. The *C<sub>max</sub>* in that study compares well with the value of 5.9 mg/L that has been reported in South Africans after an overnight fast, who also had a median AUC of 26 mg.h/L (McIlleron *et al.* 2006). Decreased bioavailability of isoniazid (and rifampicin) in some fixed dose combinations compared to single drug products has been

observed in some studies (McIlleron *et al.* 2006). On the other hand, other studies have shown bioequivalence between fixed dose combinations and the individual tablets of isoniazid, rifampicin and pyrazinamide (Agrawal *et al.* 2002), in addition to further concluding that the potential rifampicin-isoniazid interaction is clinically insignificant (Agrawal *et al.* 2004). This means that lack of bioequivalence cannot be generalized across all fixed dose combinations of antitubercular drugs and each formulation needs to be evaluated on a case by case basis.

The protein binding of isoniazid is reported to be very low, less than 10% (DrugBank 2012). Isoniazid undergoes first pass metabolism in the liver and intestines (Back and Rogers 1987), which has been shown to occur to a greater extent in fast than in slow acetylators (Weber and Hein 1979), thus differences in bioavailability between the 2 subpopulations may be expected. It is no surprise therefore that slow acetylators have higher Cmaxs compared with fast acetylators (Peloquin *et al.* 1997). Isoniazid is rapidly absorbed with a Tmax of about 1 h (Peloquin *et al.* 1997) and distributes well into tissues as evidenced by a peripheral volume of distribution reported to be as high as 1730 L (Wilkins *et al.* 2011). The half life of isoniazid in South African TB patients has been reported to be 2.8 hours (McIlleron *et al.* 2006) although up to 8 fold differences between patients has been observed. The half life would naturally be longer in slow acetylators, going up to an average of 3.4 hours in one study (Parkin *et al.* 1997), being shorter in fast acetylators where it was 1 hours in the same study (Parkin *et al.* 1997) with intermediate acetylators having a half life of 1.6 hours. Interestingly, one recent study has found that isoniazid exposure (but not the other first-line drugs) was positively correlated with CD4+ lymphocyte counts i.e. decreased AUC with decreased CD4+ counts (McIlleron *et al.* 2012). A definitive explanation for this remains to be

determined although the authors point to increased incidence of diarrhoea in patients with low CD4+ counts as a possible reason (McIlleron *et al.* 2012).

### 1.2.2.3 Isoniazid PK-PD

Isoniazid has very good EBA characterized by fast kill rates within the first few days of treatment compared to most other drugs including rifampicin (Jindani *et al.* 1980). Figure 1.3 below shows the relationship between the EBA (during the first 2 days of treatment) of various daily doses of isoniazid and rifampicin given separately as monotherapy to patients. The EBA was calculated as the mean daily difference in log CFU counts between Day 0 and Day 2 of treatment divided by 2 (Donald *et al.* 1997).

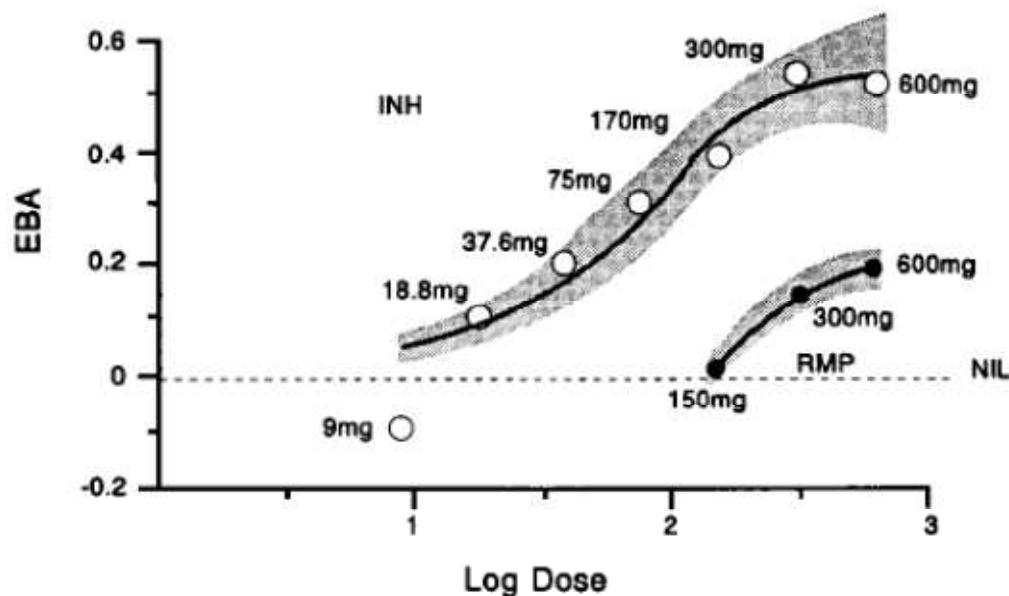


Figure 1.3: EBA of isoniazid and rifampicin with the 95% confidence intervals (shaded areas). From Donald *et al.*, 1997.

The figure shows that an isoniazid daily dose of about 300 mg results in optimal bactericidal activity, which is comparable to currently used doses clinically. Although there is no data beyond the 600 mg dose of rifampicin, it is possible, from the progression of the curve, that



600 mg is not near the maximum possible bactericidal effect of rifampicin. Several studies have shown that, similar to rifampicin, the most important predictor of isoniazid-mediated mycobacterial kill is the AUC/MIC ratio compared to C<sub>max</sub>/MIC ratios or the time above MIC (Jayaram *et al.* 2004; Gumbo *et al.* 2007; Budha *et al.* 2009) and this is in agreement with the data provided in figure 1.3.

Some studies have found that the fluoroquinolone moxifloxacin has EBA similar to that of isoniazid (Pletz *et al.* 2004) although others have shown isoniazid to be equivalent or superior to moxifloxacin depending on the method used to determine the activity (Gosling *et al.* 2003). The combination of isoniazid and moxifloxacin does not appear to have superior activity to isoniazid alone (Gillespie *et al.* 2005). Thus it is very unlikely that any other TB drug is better than isoniazid in terms of EBA particularly against rapidly dividing mycobacteria. It actually appears as though addition of any additional TB drug to isoniazid will not result in a significant increase in EBA (Mitchison 2000). However, the role of isoniazid in therapy is probably limited to these first few days of treatment apart from prevention of development of resistance. One theory was that isoniazid's activity declines because the actively dividing bacteria it kills will subsequently be non-existent and that the remaining 'persisters' belong to a different subpopulation from the outset (Jindani *et al.* 2003). Indeed, about 95% of the bacillary load in patients is eliminated in the first 2 days of therapy, predominantly by isoniazid (Mitchison 2000). However, it has subsequently been shown that the decline in isoniazid activity after 3 days is due to the development of mutations in the *katG* gene and the production of (reserpine inhibitable) efflux pumps both of which render the mycobacteria resistant to isoniazid (Gumbo *et al.* 2007). Apart from mutations in the *katG* gene, which are usually missense mutations and to a lesser extent

deletions (Heym *et al.* 1995), mutations in the *inhA* gene have also been shown to confer resistance to isoniazid.

Although it is generally accepted that the main effect of isoniazid is during the first few days of treatment, isoniazid exposure has been associated with treatment outcome. Low isoniazid plasma concentrations were found to be associated with treatment failure and even relapse after completion of treatment (Weiner *et al.* 2003). However, this particular study investigated isoniazid administered together with rifapentine once weekly in patients thus the finding may not necessarily be generalizable to daily administration of isoniazid commonly used in other TB treatment regimens.

### **1.2.3 Pyrazinamide**

Pyrazinamide remains an important part of most TB drug regimens including cases of drug resistant TB and it has been credited with shortening the duration of TB treatment due to its sterilizing activity (Grosset 1978). Pyrazinamide requires an acidic pH for its action and is much more active against old non-replicating bacilli than against growing bacilli (Zhang *et al.* 2003). This makes it a key drug in eliminating dormant bacteria infecting patients. The EBA of pyrazinamide is negligible (Botha *et al.* 1996), but its sterilizing effect begins to become apparent after about 14 days of therapy (Jindani *et al.* 2003). Pyrazinamide is a prodrug which is deaminated to the active pyrazinoic acid by bacterial nicotinamidase/pyrazinamidase. The exact mechanism of action of pyrazinamide/pyrazinoic acid remains elusive, but one possibility is that at acidic pH, pyrazinoic acid de-energizes the mycobacterial cell membrane potential which adversely affects the membrane's transport functions (Zhang *et al.* 2003). Mutations in the gene encoding the pyrazinamidase enzyme, *pncA* have been linked to resistance to pyrazinamide (Sreevatsan *et al.* 1997).

### 1.2.3.1 Pyrazinamide pharmacokinetics

The drug is rapidly absorbed with a  $T_{max}$  of less than 1 h (Peloquin *et al.* 1997) which is increased to about 3 h with administration of food (high fat meal) but without consequence on overall exposure (Peloquin *et al.* 1998). The median  $AUC_{0-\infty}$  is reported to range from 400 – 500 mg.h/L (Peloquin *et al.* 1997; McIlleron *et al.* 2006; Wilkins *et al.* 2006) with a median  $C_{max}$  of 29-53 mg/L (Peloquin *et al.* 1997; McIlleron *et al.* 2006). The pharmacokinetics of pyrazinamide has been shown to be less variable than that for the other first-line drugs (McIlleron *et al.* 2006). Pyrazinamide is reported to have an oral clearance of about 3.4 L/h and a volume of distribution of about 29 L (Wilkins *et al.* 2006) with relatively low protein binding of 10-20% (Merck 2001). It is mainly eliminated through metabolism to pyrazinoic acid by a hepatic microsomal deamidase (Lacroix *et al.* 1989) although a small amount (about 4%) is excreted unchanged via the kidneys (Ellard 1969). The part excreted through the kidneys is actually the difference between the amount filtered through the glomerulus and the amount passively reabsorbed in the tubules (Weiner and Tinker 1972) as would be expected. Pyrazinoic acid is further metabolised to 5-hydroxy-pyrazinoic acid by xanthine oxidase (Weiner and Tinker 1972; Lacroix *et al.* 1989). Pyrazinoic acid is the major metabolite and accounts for about 30% of the dose, whilst other metabolites and unchanged drug account for the rest of its elimination from the body (Ellard 1969).

### 1.2.3.2 Pyrazinamide PK-PD

The sterilizing effect of pyrazinamide is best correlated with the  $AUC/MIC$ , whilst development of resistance is linked with the time the concentration is above the MIC. An  $AUC_{(0-24)}/MIC$  ratio  $\geq 209$  in epithelial lining fluid has been proposed to result in 90% maximal bacterial killing (Gumbo *et al.* 2009). Patients with a low  $C_{max}$  of pyrazinamide

(which the authors defined as less than 35 mg/L) have been found to be at a higher risk of treatment failure or death than those with normal concentrations ( $> 35$  mg/L) (Chideya *et al.* 2009).

The graph below enhances the statement in the preceding section pertaining to the apparently delayed onset of the mycobacterial killing of pyrazinamide. Once again, this was based on the hollow fibre *in vitro* model similar to that used for rifampicin and isoniazid.

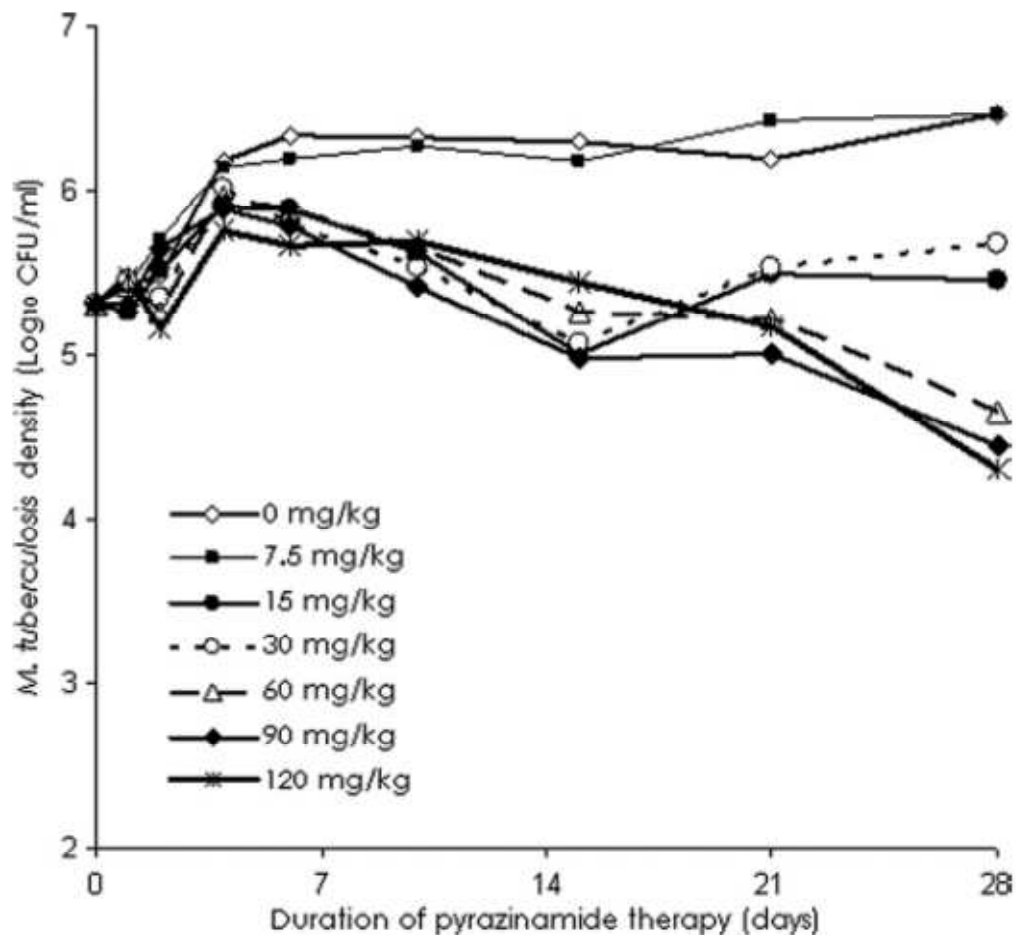


Figure 1.4: Graph showing decline in *M. tuberculosis* with duration of pyrazinamide administration. From Gumbo *et al.*, 2009.

Regardless of the dose, there is an upward trend, showing growth of *M. Tuberculosis* similar to the control of 0 mg/kg for the first week or so of therapy, before the decline to levels lower than the baseline are evident after about 2 weeks.

### **1.2.4 Ethambutol**

The main role of ethambutol in first-line TB treatment regimens is to prevent emergence of rifampicin resistance when resistance to isoniazid may be present (Blumberg *et al.* 2003). Microbial killing of ethambutol is exposure dependent (AUC/MIC driven) while suppression of efflux pump mediated resistance best correlates with the time the drug concentration is above the MIC (Srivastava *et al.* 2010). Half maximal killing occurs at a  $AUC_{(0-24)}/MIC$  ratio of 79 (Srivastava *et al.* 2010). In addition to the efflux pump mechanism of resistance to ethambutol, some genetic mutations have also been described as other mechanisms of resistance (Sreevatsan *et al.* 1997).

#### **1.2.4.1 Ethambutol pharmacokinetics**

Ethambutol is absorbed rather slowly with a  $T_{max}$  of 2.5 h when administered on an empty stomach (Peloquin *et al.* 1999). Food increased this  $T_{max}$  to 3.2 h and also resulted in reduced  $C_{max}$  (from a mean of 4.5 mg/L to 3.8 mg/L), but with a negligible effect on the total exposure i.e.  $AUC_{0-\infty}$  which was about 29 mg.h/L (Peloquin *et al.* 1999). Other studies have reported a similar  $AUC_{0-\infty}$  which was between 20-30 mg.h/L for doses between 800 – 1200 mg (Jonsson *et al.* 2011). Interestingly, absorption of ethambutol has been shown to be erratic and to be less complete in children compared with adults (Zhu *et al.* 2004).

A pharmacokinetic study conducted in South African adult patients reported ethambutol to have a plasma clearance of about 40 L/h, a central volume of distribution of 82 L and a

peripheral volume of 623 L (Jonsson *et al.* 2011). A high intercompartmental clearance of 34 L/h coupled with the high volume of distribution (Jonsson *et al.* 2011) suggests that ethambutol distributes rapidly and well into tissues. Body weight has been found to be a significant predictor of ethambutol clearance and volume of distribution (Jonsson *et al.* 2011) meaning that overweight or obese patients will have lower drug concentrations than leaner patients which may be of clinical significance (Hall *et al.* 2012). HIV infection has also been reported to result in slightly lower ethambutol drug concentrations (Zhu *et al.* 2004; Jonsson *et al.* 2011). Ethambutol is reported to be about 20-30% bound to plasma proteins (Lee *et al.* 1977).

### **1.2.5 Ofloxacin**

The fluoroquinolones including ofloxacin, moxifloxacin, levofloxacin and to a lesser extent gatifloxacin are found in many regimens used to treat MDR-TB but have also been investigated in regimens for ordinary drug sensitive TB as part of attempts to shorten treatment by up to 2 months (Nuermberger *et al.* 2004; Nuermberger *et al.* 2004; Rustonjee *et al.* 2008). For ordinary TB, they are also part of attempts to reduce pill burden by allowing less frequent drug administration to once or twice per week (Zvada *et al.* 2012) instead of the current daily dosing, or simply part of efforts to try to improve cure rates. Due to its relatively low cost and widespread availability, ofloxacin has been the fluoroquinolone of choice in South African MDR-TB programmes (Health\_and\_Medical\_Publishing\_Group 2010). This is unfortunate because ofloxacin is known to be less potent than the other fluoroquinolones (except ciprofloxacin) both *in vitro* and *in vivo* (Hu *et al.* 2003; Rustonjee *et al.* 2008).

### 1.2.5.1 Ofloxacin pharmacokinetics

Ofloxacin is reported to have an excellent absorption profile, with more than 95% of the oral dose reaching systemic circulation, and the C<sub>max</sub> being achieved just over an hour after dose administration (Lode *et al.* 1987). However, delayed absorption has been reported and this may be due to food effects (Stambaugh *et al.* 2002). Food does not affect the overall drug exposure although the T<sub>max</sub> may be longer (Janssen-Ortho 2006). The C<sub>max</sub> and AUC<sub>0-24</sub> following an 800 mg dose are about 9 mg/L and 100 mg.h/L respectively (Stambaugh *et al.* 2002). The drug is about 25% protein bound and its elimination from the body is predominantly via renal mechanisms with only 4% being metabolised (Lode *et al.* 1987) and another 4-8% appearing in faeces, suggesting a small extent of biliary excretion (Janssen-Ortho 2006). As would be expected, renal function as determined by creatinine clearance, significantly influences the rate of elimination of ofloxacin (Stambaugh *et al.* 2002) meaning that doses may need to be reduced in patients with renal impairment. The clearance and volume of distribution of ofloxacin in a typical 70 kg individual would be about 8.4 L/h and 90 L respectively, resulting in an elimination half life of about 7.4 h (Stambaugh *et al.* 2002).

### 1.2.5.2 Ofloxacin PK-PD

The mechanism of action of ofloxacin (and other fluoroquinolones) is inhibition of bacterial DNA gyrase (topoisomerase IV), which is essential for bacterial DNA replication and growth (Sato *et al.* 1986). Mutations in the genes encoding for the DNA gyrase subunits A and B (*gyrA* and *gyrB*), particularly *gyrA*, have been shown to result in resistance to fluoroquinolones (Kocagoz *et al.* 1996). The best predictor of *in vivo* efficacy for this class of drugs is the AUC/MIC (Shandil *et al.* 2007). Several studies have shown that the optimal kill rates and low probability of development of resistance will occur when the AUC/MIC ratio for the fluoroquinolones is at least 100 (Ginsburg *et al.* 2003; Schentag *et al.* 2003; Schentag

*et al.* 2003). The EBA of an 800 mg dose ofloxacin is reported to be about half that of a 300 mg dose of isoniazid (Sirgel *et al.* 2000). The graph below (figure 1.5) illustrates the effect of increasing exposure of ofloxacin on bacillary load in the lungs of mice after 4 weeks of treatment.

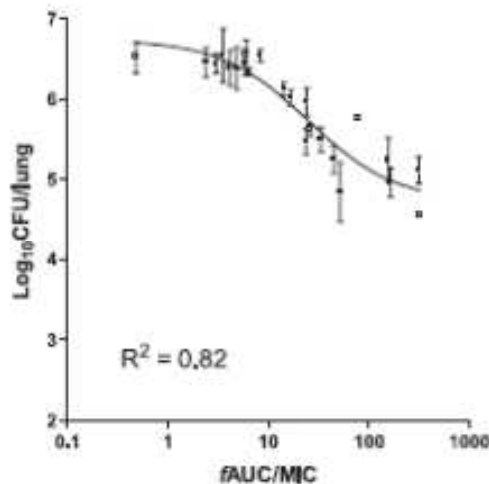


Figure 1.5: Relationship between ofloxacin exposure and amount of *M. tuberculosis* in lungs of mice. From (Shandil *et al.* 2007)

Figure 1.6 below shows that ofloxacin (shown as round open circles) is less potent than most quinolones but also that at expected clinical exposures following an 800 mg dose which produce an average maximum concentration of about 9 mg/L (Stambaugh *et al.*, 2002), the optimal bactericidal effect of ofloxacin is not realised. The graph shows *in vitro* experiments where *M. Tuberculosis* was incubated with various quinolones over a 5 day period before counting of the bacteria was undertaken.



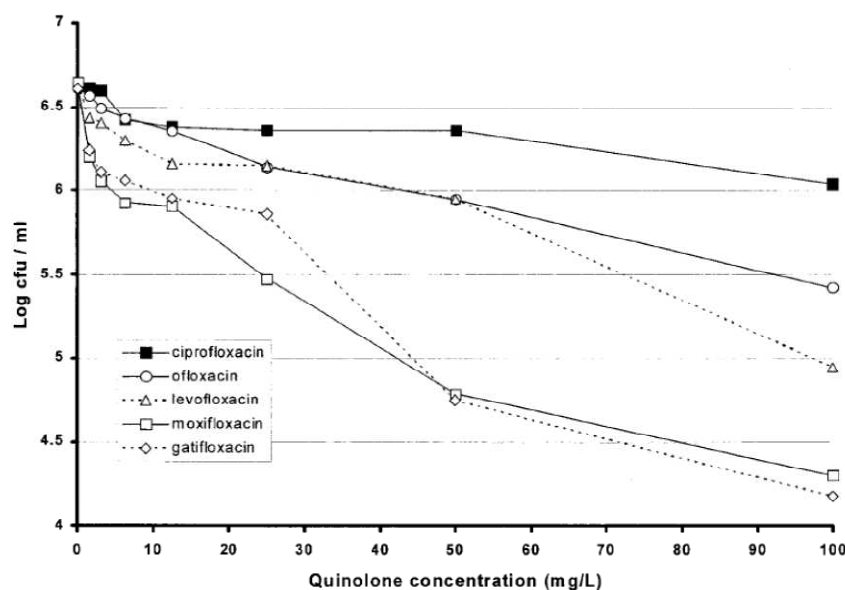


Figure 1.6: Viable counts after exposure of *M. tuberculosis* to various fluoroquinolones. From (Hu *et al.* 2003).

### 1.3 Determination of the efficacy of antitubercular drugs

The term ‘efficacy’ was chosen over the more commonly used term ‘bactericidal activity’ because the following discussion aims to encompass bactericidal effects based on kill rates of the different bacillary subpopulations, but in the light of ultimately translating this to treatment of patients in routine clinical settings. TB most frequently affects the lungs, although extrapulmonary TB affecting pleural sites, lymph nodes, bone, meninges or any other organ is not uncommon (Mehta *et al.* 1991), and may actually be increasing in proportion to pulmonary TB (Garcia-Rodriguez *et al.* 2011). The work in this thesis therefore focuses on pulmonary TB which is the most common. A variety of studies investigating the response of patients to different kinds of drugs regimens and doses have been carried out over the last decade or two. A number of different endpoints and markers have been investigated in the conduct of these studies as will be discussed in section 1.3.4. A limitation of using measures of drug exposure based on plasma drug concentration-time profiles immediately becomes apparent since for (pulmonary) TB, the site of action is in the lung where the bacilli

reside. Differences between the plasma drug concentration and the concentration at the site of action can be expected, although the two can be correlated (Kjellsson *et al.* 2012). Depending on the drug penetration properties, higher, lower or similar concentrations in the lung compared to plasma can be achieved. Until reliable and robust methods of determination of drug concentrations at the site of action become available, plasma drug concentrations will continue to be used as a proxy for what is happening downstream. The same can be said for sputum and the extent to which it is a representation of what is happening in other parts of the lung where the majority of bacilli are expected to be residing such as granulomas and other lesions (Joel *et al.* 2012). One other limitation of using sputum as a measure of bacillary load is variable consistency and quality of the sputum since it can easily be diluted with saliva and is often difficult to expectorate, thus inflating variability between and within patients. However, almost all the TB studies to date have used measures of bacillary load based on sputum samples and this is unlikely to change.

### **1.3.1 Colony forming unit counting**

CFU counts are a common way to determine the concentration of bacteria from a clinical sputum specimen. The sputum specimen is then usually diluted before inoculating a known volume onto a solid (usually agar) culture medium and incubating for a period of time. The assumption is that one visible colony arises from the growth of one viable bacterium hence the phrase ‘colony forming unit’ (Todar 2012). Serial dilutions are usually performed such that the numbers of colonies to count on the plate are reasonable although this does not always happen, leading to situations such as ‘too-numerous-to-count’ (TNTC). This can cause problems of introducing bias in the data since data that is TNTC is often discarded, although some (complex) mathematical techniques have now been developed to deal with such situations (Haas and Heller 1988; Blodgett 2008).

Another problem with CFU counting is that it is labour intensive, costly and technically challenging, requiring skilled microbiologists. However new automated CFU counting procedures are now being developed (Brugger *et al.* 2012). Clumping, or aggregation of several individual bacteria into a single CFU is a possible occurrence that may result in underestimation of the actual number of bacteria present in the initial inoculum (Pathak *et al.* 2012). CFU counting is also unable to distinguish between differing metabolic activities of the subpopulations of mycobacteria i.e. whether or not they are fast or slow replicating. Whilst the colonies may be of different sizes and dimensions, they are still counted as single units.

Focusing now on mycobacteria, the most common solid media used is the egg-based Löwenstein-Jensen (LJ) medium which is the first choice for culture of clinical samples, although the agar-based Middlebrook 7H10 and 7H11 media are sometimes used (Saito 1998). In spite of the drawbacks of CFU counting it has been in use for decades and is still used as the gold standard of measuring response to treatment in almost all studies investigating pharmacodynamic activity of antitubercular drugs both *in vitro* and *in vivo* particularly in EBA studies (Gosling *et al.* 2003; Gillespie *et al.* 2005; Gumbo *et al.* 2007; Gumbo *et al.* 2007). CFU counts remain a reliable and useful measure of bacillary decline and have shown expected trends of decline from high values before patients start treatment, to low values as drug therapy continues (Diacon *et al.* 2007).

### **1.3.2 The emerging era of liquid culture and time to detection**

In chronic TB infection, whereby some of the bacilli are expected to be dormant, it has been shown that some mycobacterial isolates will grow in liquid, but not in solid media (Dhillon *et*

*al.* 2004). The converse is unlikely to be true as other studies have found significantly lower recovery rates of *M. Tuberculosis* isolates in solid LJ plates compared to liquid-based media (Chien *et al.* 2000; Somoskovi *et al.* 2000). The CFU method may therefore underestimate the total mycobacterial load in chronic infection (Pathak *et al.* 2012). This means that the best representation of the dynamics of *M. Tuberculosis* in patients on TB therapy would be results from liquid culture rather than solid culture especially when considering the importance of elimination of bacteria that are not dividing rapidly and therefore the sterilizing activity of drugs. On the other hand, when one wishes to investigate the activity of a drug mainly on rapidly dividing organisms in the early days of treatment, solid culture may be a better method since it may be more sensitive to changes in the rapidly dividing population.

Mycobacteria growth indicator tubes (MGIT) are a liquid culture based system comprised of modified Middlebrook 7H9 broth. The means of detection of mycobacteria is based on a silicon rubber disk impregnated with ruthenium pentahydrate which is placed at the bottom of the tube containing the broth. Ruthenium pentahydrate fluoresces naturally but this fluorescence can be quenched by oxygen. Therefore, if mycobacteria are present and growing, they will consume the oxygen in the MGIT until it is no longer sufficient to quench the fluorescence of ruthenium pentahydrate and this is then interpreted as a positive test result for TB (Chew *et al.* 1998). Before inoculating the tube with the specimen for testing, the microbiologist first needs to decontaminate the specimen (usually sputum) to make sure that only mycobacteria and not other microorganisms which can potentially use oxygen are present. The usual decontamination procedure is *N*-acetyl-L-cysteine-2% sodium hydroxide, followed by a battery of antibiotics (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) (Chang *et al.* 2002). A fully automated MGIT culture system is commercially available (BACTEC MGIT 960 from Becton Dickinson Microbiology systems)

which can test up to 960 MGIT vials simultaneously and will automatically give out a reading of when a positive test result was encountered for each tube. Absence of a positive result after 42 days of incubation is considered a negative result. This relatively long incubation time enables the detection of bacteria that may be growing slowly, or that may have been a very small inoculum number at the beginning. The time to detection (TTD) of a positive result is significantly shorter by about 50% than LJ media (Somoskovi *et al.* 2000), although MGIT systems generally have a slightly higher contamination rate compared to LJ media (Otu *et al.* 2008). Since the TTD takes a much shorter time, it is of benefit to patients and clinical teams as the time to a positive or negative result is shorter compared to solid media.

The short TTD of positive results in MGIT, coupled with its ability to detect the presence of slow growing or dormant mycobacteria (which would otherwise be false negatives on solid media) make it an attractive option for routine clinical practice as well as for studies that aim to investigate pharmacodynamics of antitubercular drugs. Not surprisingly, TTD has been proposed as an alternative to CFU counting (Pheiffer *et al.* 2008). Longer TTD would be associated with lower initial bacillary burden (and therefore CFU count) although the 2 measures appear to be weakly correlated and no quantitative relationship has been described (Pheiffer *et al.* 2008). Another possible advantage of TTD in liquid culture is that due to its sensitivity, it is likely to give positive results later on in studies following patients up to 8 weeks when solid media is more likely to give negative (thus censored) results, hence TTD will have more data points available for the statistical analysis thereby increasing study power (Diacon *et al.* 2011). In fact, it has been shown that the proportion of patients with negative sputum culture results after 8 weeks of standard treatment is significantly higher when solid media are used than when the same sputum is cultured in liquid media ( $p < 0.01$ ,

(Dorman *et al.* 2009)). TTD is expected to be longer for slow-growing bacteria compared to rapidly dividing organisms which would be consuming more oxygen.

What is even more interesting from Dorman *et al.*'s study is that only 41% of week 8 cultures from patients from African sites (South Africa and Uganda) were negative in liquid media compared with 91% in solid media ( $p < 0.01$ ). Yet in the same multi-centre study, negative week 8 sputum results from non-African sites (predominantly USA) were 73% (liquid media) and 81% for solid media and were not significantly different from each other (Dorman *et al.* 2009). An explanation for such a discrepancy has not been found, but it may be due to the fact that patients from the African sites in the study had double the prevalence of lung cavities compared to patients from non-African sites in that study (Dorman *et al.* 2009) and thus had higher initial bacillary loads (Perrin *et al.* 2010) of dormant bacteria which may only grow in liquid media. Other possible reasons may be differences in strain and subtype of *M. tuberculosis* between the populations.

### **1.3.3 Other methods of detection of TB**

Currently the most common method of detection of TB in sputum from patients is smear microscopy in which mycobacteria are observed in processed sputum sample under a microscope (WHO 2012). This method is not very sensitive and captures only about 56% of cases of pulmonary TB although it is the main method in low to middle income countries which do not currently have capacity for other methods (WHO 2012). In absence of culture-based methods, a diagnosis of TB even when the sputum smear is negative can be made based on clinical and radiological evidence (Siddiqi *et al.* 2003). The WHO has recently recommended nucleic acid amplification tests, notably the Xpert<sup>®</sup> MTB/RIF assay for rapid detection of TB in sputum using the GeneXpert platform (Boehme *et al.* 2010). This test is

reported to be as sensitive as solid culture methods (WHO 2012) although costs for implementation in low to middle income countries remain a concern (Vassall *et al.* 2011).

### **1.3.4 Types of antitubercular drug studies in patients**

#### **1.3.4.1 Evaluation of response**

The gold standard efficacy endpoint of phase III TB drug trials is cure upon treatment completion (at least 2 consecutive negative sputum culture results, most often in solid media) and thereafter incidence of relapse over the next 1-2 years after treatment completion (Ginsberg and Spigelman 2007). Relapse is defined as the case whereby a patient becomes culture negative for *M. Tuberculosis* during treatment but then turns positive again at some point within 1-2 years after completion of treatment (Chang *et al.* 2004). Caution is required when distinguishing between recurrence of a TB infection due to relapse or whether it is due to reinfection. DNA fingerprinting can help to identify the strain of the mycobacteria and thereby distinguish between the 2 scenarios (Sonnenberg *et al.* 2001).

Since the treatment of TB is relatively long (at least 6 months) and the time to investigate relapse is a further 1-2 years, surrogate endpoints have been investigated in an attempt to be able to conduct feasible and realistic studies as following up patients for long periods is associated with increased study costs and feasibility problems such as patient dropout and increased amounts of missing data. A negative sputum culture result after 8 weeks of treatment is widely accepted as a surrogate endpoint for sterilizing activity (Mitchison 1993). It is therefore not surprising that there are numerous clinical studies that have followed patients over a 2-month period (which also happens to be the duration of the intensive phase

of TB treatment) investigating various antitubercular drug regimens (Burman *et al.* 2006; Rustomjee *et al.* 2008; Dorman *et al.* 2009).

The use of the 2 month sputum conversion status is not without problems. For example, the study conducted by Burman *et al* found no difference between proportions of patients who had been given moxifloxacin and those who received ethambutol (in addition to the standard drugs, rifampicin, pyrazinamide and isoniazid) (Burman *et al.* 2006). However, a closer look at the results demonstrate that patients who received moxifloxacin had a shorter time to culture conversion thus moxifloxacin would have been a better drug when investigating treatment regimens of shorter durations (Perrin *et al.* 2007). This suggests that the 2 month ‘snapshot’ is less informative than the more continuous measure of time to culture conversion. Sadly, in most studies, the primary method of analysis is currently the comparison of proportions of negative sputum cultures at 2 months. An even better method of investigating antitubercular drug activity in clinical trials is a non-linear mixed effects (NLME) modeling approach to analysis of serially (weekly) collected sputum CFU counts over a 2 month period (Davies *et al.* 2006). However the best way to design such studies is currently unknown and the design of the study may result in different study outcomes. For example, the NLME method of analysis was implemented by Rustomjee *et al*, who fitted CFU count data from patients followed over a 2 month period, with weekly sputum CFU counts in a Phase II study investigating the sterilising activities of fluoroquinolones (Rustomjee *et al.* 2008). It is interesting to note that Rustomjee *et al* found a statistically significant increase in the kill rate for the moxifloxacin-containing regimen using the NLME approach, whilst two similar studies with even larger numbers of patients failed to find any significant benefit of moxifloxacin after analyzing the 2 month culture ‘snapshot’ results



(Burman *et al.* 2006; Dorman *et al.* 2009). It must be emphasized once again that all of these studies involved culturing *M. Tuberculosis* on solid media.

Studies investigating EBA of one regimen/drug versus another are often the first step in evaluating pharmacodynamic activity of drugs in patients before the longer studies discussed above are carried out. The selection of drugs for later trials is then based upon results of these studies. EBA is usually calculated as the rate of decline of the logarithm of the bacterial CFU counts within a given period e.g. the first two days of treatment or between Day 2 and Day 8 of treatment. Between Day 2 and Day 8, the overall bactericidal activity of a multidrug regimen is generally less than that for the first two days (Brindle *et al.* 2001; Gillespie *et al.* 2002). The typical duration of such studies is between 2 and 14 days and they usually have intensive sputum sampling for determination of the CFU counts (Chan *et al.* 1992; Sirgel *et al.* 1993; Botha *et al.* 1996). Importantly, most of the studies investigating single drugs have durations up to 14 days. The risk that monotherapy may potentially result in drug resistance (Gumbo *et al.* 2007) raises ethical concerns and limits the frequency and duration of such studies.

Whilst EBA studies are common and have their usefulness, results from such studies must be interpreted with caution. Firstly, as mentioned above, addition of a drug to an isoniazid-containing regimen is unlikely to show any improvement in EBA, no matter how good the investigational drug may be. Secondly, the lack of EBA does not mean the drug will be useless in TB treatment, since sterilizing activity is ultimately what will determine whether or not a patient is cured. This is certainly the case with pyrazinamide and aminoglycosides (Jindani *et al.* 1980; O'Brien 2002). It also does not follow that a drug with good EBA will be a good sterilizing drug.

#### 1.3.4.2 Study design

Randomised controlled trials (RCT's) are considered the most rigorous and important way to evaluate whether or not an intervention (often a drug regimen) is associated with a particular outcome (Sibbald and Roland 1998). Numerous double-blind placebo-controlled trials have been conducted for many years but as drug therapies now exist for many diseases and conditions (including TB), it may become necessary to compare the new drug with what is currently on the market in randomized active-controlled trials (Gomberg-Maitland *et al.* 2003) rather than in placebo-controlled trials. For trials investigating the test drug regimen compared with another active control, superiority trials are the most common whereby the aim is to show that the new treatment is superior to the current standard and therefore reject the null hypothesis that there is no difference between the two (Christensen 2007), similar to placebo-controlled trials. This null hypothesis is reversed in equivalence trials which aim to show that the new therapy is equivalent in efficacy to the active control, but may have other advantages related to convenience of drug administration or less side effects or lower cost etc. (Jones *et al.* 1996). In equivalence trials, the null hypothesis is that a minimum (prespecified) difference between the two therapies exists and the aim of the trial is to disprove this in favour of the alternative hypothesis which is that there is no difference and usually requires a larger sample size than superiority trials (Jones *et al.* 1996; Christensen 2007). A non-inferiority trial is similar to an equivalence trial, but has a one-sided analysis in that the aim is to show that the efficacy of the new regimen is no less, rather than to show that it can be greater than or less than the active control i.e. the new treatment is no worse than current therapy (Christensen 2007). Non-inferiority trials must be carefully designed to avoid the possibility of declaring a new regimen to be non-inferior simply because it was compared

with a regimen that was already inadequate (Nunn *et al.* 2008). Equivalence trials are rare because most such studies are designed as non-inferiority trials (Piaggio *et al.* 2006).

Although they are mostly RCTs, the above terminology does not seem to have been used for most antitubercular drug studies, but it appears that many of the studies have been superiority trials, especially EBA studies. However, some of the current aims in TB drug development are to shorten the long duration of current regimens (Nueremberger *et al.* 2004) and/or reducing pill burden thereby increasing adherence through intermittent drug administration (Zvada *et al.* 2012), some of these previous studies may be considered equivalence or non-inferiority studies.

## 1.4 Pharmacometrics

The FDA has defined pharmacometrics as a branch of science that quantifies drug, disease and trial information to aid efficient drug development and/or regulatory decisions. Drug models describe the relationship between exposure (or pharmacokinetics), response (or pharmacodynamics) and individual patient characteristics. Disease models describe the relationship between biomarkers and clinical outcomes, time course of disease and placebo effects. The trial models describe the inclusion/exclusion criteria, patient discontinuation and adherence (FDA 2010). The field arose from population pharmacokinetics which employs a NLME modeling approach to data analysis, whereby population mean kinetics, inter- and intra-individual variability and measurement error are quantified (Sheiner and Beal 1980). This approach has been extended to include pharmacodynamics, such that the PK-PD relationship estimated. This population modeling approach is especially beneficial when data is sparse, and has been shown to be a better data analysis method compared to other methods

such as the standard two stage approach or the naive pooled method (Sheiner and Beal 1980; Sheiner and Beal 1983).

The terms pharmacometrics and ‘modeling and simulation’ are used interchangeably, although modeling most often refers to the process of building (mathematical and statistical) models from the data, whilst simulation then refers to the use of the final model to predict new data, usually under different circumstances. Modeling and simulation has been shown to be an important development towards improvement of decision making in the pharmaceutical industry (Gieschke and Steimer 2000; Miller *et al.* 2005). The increased use of modeling and simulation as part of drug development is expected to lead to fewer drug failures and smaller numbers of studies before a drug succeeds in a new drug application (Rajman 2008).

#### **1.4.1 Clinical Trial Simulation**

A more recent application of pharmacometrics has been in the area of clinical trial simulation. Clinical trials have been the standard method of evaluating the safety and effectiveness of new drugs in human beings. For TB, since standard approved therapy is existent in most settings, the traditional study design has been the addition of the trial drug to standard therapy in RCTs (Diacon *et al.* 2009), or substitution of one of the standard antitubercular drug with the investigational drug (Bock *et al.* 2002; Burman *et al.* 2006; Rustomjee *et al.* 2008; Dorman *et al.* 2009) in parallel group studies. The endpoints of cure upon treatment completion (or at the end of the investigational period) or relapse are binary (yes or no) outcomes. Whilst use of these endpoints may answer the question at hand, they may be an expensive and less informative way to do so, requiring larger sample sizes than NLME modeling based methods as described above. Introduction of a component of time i.e. time to cure or culture conversion, is expected to be more informative as found by Dorman *et*

*al* (2009). The authors found no difference in cure rates of a regimen with moxifloxacin compared with standard treatment when using the 2 month sputum conversion endpoint. However, when looking at the time at which negativity was attained (which is not necessarily 2 months), they found that the moxifloxacin-containing arm resulted in earlier attainment of sputum culture negativity.

The duration of a clinical study is very important as one may not find a significant effect of a drug if the trial period is too short as has been previously demonstrated with study periods limited to 8 days (Jindani *et al.* 2003). In addition, the sampling times at which sputum is collected for investigation of pharmacodynamic effects are important (Davies *et al.* 2006). When designing a longer study to investigate the effect of a slow acting drug, one must consider issues such as missing data, decreased patient adherence, patient dropout, and protocol deviations (Holford *et al.* 2000; Holford *et al.* 2010), become important and will ultimately have a significant influence on power of the study. All of the above means that there are several considerations when designing a study to investigate the effect of a new drug.

Clinical trial simulation can be used to test how different study designs and factors can affect the outcome of a study and thus identify the best clinical trial design. The study factors include the nature of the disease and the pharmacokinetics and pharmacodynamics of the drug (Lockwood *et al.* 2006) whilst practicalities such as protocol deviations also need investigation (Girard 2005). Clinical trial simulation allows us to answer several ‘what if’ questions, thereby increasing the probability of success of a clinical trial (Bonate 2000). Some ‘what if’ questions include: how will a change in inclusion criteria affect outcome, or what is the effect of a 10% decrease in patient adherence (Bonate 2000)? When an

antitubercular drug fails a clinical trial, it may be because the drug does not work, or the study design was not optimal e.g. wrong group of patients, or wrong dose or low statistical power (Bonate 2000).

## 2. PROBLEM STATEMENT AND JUSTIFICATION

A brief summary of the literature review above follows before a discussion of the individual components of the project.

The standard regimen for TB in South Africa, and many parts of the world is comprised of rifampicin, isoniazid, pyrazinamide and ethambutol. Generally, studies in African patients are few in spite of the fact that there is a high TB burden on the continent. In addition, findings in the studies conducted in other parts of the world may not be generalizable to Africans due to potential differences between the mycobacterial strains found in Africans and those in other parts of the world, the high HIV burden on the continent resulting in differences in patient response and potentially antibiotic pharmacokinetics, the use of different fixed-dose combinations and different clinical settings in general. The pharmacokinetics of these first-line drugs in South Africans have been described by McIlleron *et al* (McIlleron *et al.* 2006; McIlleron *et al.* 2007; McIlleron *et al.* 2009) using non-compartmental analysis (NCA) based methods. NCA methods have the drawback of requiring intensive pharmacokinetic sampling in patients, which comes at a financial and ethical cost. They also have limited usefulness when it comes to extrapolations for other situations e.g. dose adjustment. The pharmacometric (modeling and simulation) approach has gained momentum globally over the last decade or so and the field is changing rapidly. Pharmacometric analyses of first-line TB drug pharmacokinetics in South Africans has been performed by Wilkins *et al* (Wilkins *et al.* 2006; Wilkins *et al.* 2008; Wilkins *et al.* 2011) but there is not much outside of these studies. There is a need to confirm the findings of Wilkins *et al* in different patient cohorts (that may have different demographics and may be on different drug formulations), coupled with the fact that modeling and simulation technologies have been rapidly advancing over the years,

enabling more robust analyses. Furthermore despite the important role of fluoroquinolones in MDR-TB and their widespread use, there have been no studies on their pharmacokinetics in the South African patient population.

Of the 4 first-line drugs, rifampicin is considered the most important because of its ability to kill both rapidly dividing mycobacteria and less metabolically active bacteria. In spite of its importance in the regimen, known high interpatient variability, and the known mechanisms of rifampicin disposition in the body, the pharmacogenetic determinants of rifampicin pharmacokinetics have hardly been described, unlike the popular isoniazid and NAT-2-mediated acetylation.

Whilst the need for robust pharmacometric analyses of the antitubercular drugs used in South Africans has been highlighted above, the pharmacokinetics of the drugs must be viewed in light of their expected pharmacodynamic effects in patients. The adequacy of the drug concentrations needs evaluation to determine whether or not there exists a need for dose adjustment. For drugs like rifampicin, a minimum  $C_{max}$  of at least 8 mg/L has been proposed (Peloquin 2002), and for fluoroquinolones, minimum AUC/MIC ratios have been suggested. The pharmacodynamic effects of the drugs in patients on treatment need evaluation. Whilst this has been done for CFU counting on solid media, the pitfalls of CFU counting have been discussed, and liquid culture in MGIT is increasingly becoming common in the routine clinical setting. However a pharmacodynamic model based on liquid culture systems for TB is currently not available. Such a model would then enable investigation of the effect of the pharmacokinetics of the drugs on the rate of decline of bacilli in sputum based on increase in TTD with time on treatment.



Finally, it is of limited use to accurately describe drug pharmacokinetics and make suggestions for new dosing regimens or drug combinations without developing efficient ways of testing these changes in patients through clinical trials. Modeling and clinical trial simulation can help identify and refine the optimal study design to test the effect of one drug regimen compared to an alternative.

## **2.1 Pharmacokinetics and pharmacogenetics of rifampicin**

Several studies have reported rifampicin, the most important first-line drug in the treatment of TB, to have highly variable pharmacokinetics (Peloquin *et al.* 1997; McIlleron *et al.* 2006; Wilkins *et al.* 2008). The metabolic pathways of rifampicin have been poorly understood until recently. Rifampicin is known to be a substrate of drug transporters p-glycoprotein (Schuetz *et al.* 1996) and OATP-C (Kim 2003; Tirona *et al.* 2003). Recent studies have found rifampicin concentrations to be lower in African patients than non-African patients (Weiner *et al.* 2010). Other multi-centre clinical studies have found lower sputum culture conversion rates in patients from African sites compared with patients from non-African sites (Burman *et al.* 2006; Dorman *et al.* 2009). To our knowledge, a pharmacogenetic basis of low rifampicin plasma concentrations in African patients has not been reported.

## **2.2 Pharmacokinetics and pharmacodynamics of ofloxacin in multi-drug resistant tuberculosis**

Fluoroquinolones are a key drug class in the treatment of MDR-TB and have been found to improve cure rates of MDR-TB (Ginsburg *et al.* 2003; Migliori *et al.* 2008). The PK-PD index that best correlates with fluoroquinolone activity is the AUC/MIC ratio. A ratio of at least 100 is required for optimal kill and prevention of emergence of resistance (Shandil *et al.*

2007; Schentag *et al.* 2003). In South Africa, ofloxacin is the fluoroquinolone most widely used for MDR-TB, although nothing is known about its pharmacokinetics in Africans. The distribution of ofloxacin MICs in South African patients with MDR-TB is also not known. In addition, *M. tuberculosis* susceptibility to ofloxacin is known to be reduced in the presence of resistance to rifampicin (Louw *et al.* 2011), which by definition occurs in all our patients with confirmed MDR-TB.

### **2.3 Analysis of time to detection data as a pharmacodynamic marker**

TTD in liquid culture is more sensitive and takes a shorter time to record a positive result than solid media, therefore it is a better method for detection of *M. tuberculosis* in patients' sputum. However, there is a need for a method that enables proper quantitative analysis of this kind of data. The MGIT test was not developed with a quantitative basis in mind, but was developed to shorten the time to get a result for presence or absence of TB. Given that MGIT results are becoming increasingly available in clinical studies, and routine clinical settings, there is a need to develop a method of analysing data comprised of TTD. This would enable TTD to be used as a marker for pharmacodynamic activity for comparison of different regimens or interventions in a quantitative and scientific manner.

### **2.4 Effect of antitubercular drug exposure on rate of kill of the bacilli *in vivo***

There are *in vitro* and animal data showing exposure-response relationships of the antitubercular drugs (Gumbo *et al.* 2004; Gumbo *et al.* 2007; Shandil *et al.* 2007). However, clinical studies in humans have been limited to a comparison of regimens or comparison of different doses within a regimen (Dietze *et al.* 2008; Rustonjee *et al.* 2008; Dorman *et al.* 2009) with just a handful of real exposure-response relationships being described (Weiner *et al.* 2003; Weiner *et al.* 2005).

## **2.5 Use of clinical trial simulation to improve design of antitubercular drug studies**

The 2 month surrogate endpoint in TB drug trials (at least for phase II studies) seems to be a rule of thumb suggested by one individual 20 years ago (Mitchison 1993). We are not aware of any researchers that have attempted to investigate whether it is really the optimal time point in which to compare the new regimen under investigation with the standard treatment, especially since regimens may change in potency over time. It is unknown whether following patients for slightly longer or slightly shorter periods would result in altered study power and/or conclusions from the study. As aforementioned, when a drug fails a trial, it could be due to the fact that the drug in fact does not work, or it could be a poor study design (e.g. too few study participants, study duration was suboptimal) or even due to a less informative approach for the data analysis. The NLME modeling approach has been shown to be the ideal method of data analysis of serial log CFU counts from patients (Davies *et al.* 2006). However, the numbers of patients and optimal study design for a typical TB drug trial intending to use NLME for data analysis has not been critically evaluated.

### 3. AIMS

The purpose of this work was:

1. To describe the population pharmacokinetics of the first-line antitubercular drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) in South African patients and determine factors influencing interindividual variability. Using more advanced modeling methodologies, this aims to improve upon the work done by McIlleron *et al* and Wilkins *et al* in different patient populations. Specifically for rifampicin, a key drug in the regimen, to determine the effect of genetic polymorphisms influencing the expression and/or activity of drug transporters on the pharmacokinetics of rifampicin. Pharmacogenetics has the potential to explain some of the high variability previously reported by McIlleron *et al* and Wilkins *et al* whose studies did not include pharmacogenetic analyses.
2. To describe the population pharmacokinetics of ofloxacin in South African patients with MDR-TB and evaluate the adequacy of the ofloxacin exposure in light of the susceptibility of the *M. tuberculosis* strains in this patient population through estimating the probability of achieving a PK-PD target based on the AUC/MIC ratio.
3. To develop a quantitative time to event pharmacodynamic model for the analysis of TTD data in liquid culture obtained from patients on first-line antitubercular drugs being treated for TB.
4. To determine the effect of drug exposure of the individual first-line drugs on rates of kill of the bacteria in patients on treatment and evaluate the effect of individual drugs in the multi-drug regimen on patient outcomes.
5. Using clinical trial simulation, to determine the optimal design, duration and number of patients for a TB drug study investigating the sterilizing activity of a (hypothetical) new drug taking into account realistic clinical study settings.

## 4. METHODS

The general methods for the various studies are outlined below, with more specific issues being found in the appropriate sections of the following chapters.

### 4.1 Population pharmacokinetics of first-line drugs in South African patients with tuberculosis

This was a pharmacokinetic sub-study of a randomized controlled trial investigating the effect of vitamin A and zinc supplementation on treatment outcomes in South African patients with TB. The parent study was conducted by Visser *et al* (2010) and enrolled 154 patients who were randomly assigned to receive vitamin A plus zinc supplementation or placebo, in addition to the standard first-line TB treatment regimen with dosages in accordance with table 1 below:

Table 4.1: Daily drug dosages of first-line drugs in South African adult patients with TB\*

Drug	Average dose (mg/kg)	Dose in mg for each weight band			
		30-37 kg	38-54 kg	55-70 kg	>70 kg
Rifampicin	10	300	450	600	750
Isoniazid	5	150	225	300	375
Pyrazinamide	25	800	1200	1600	2000
Ethambutol	15	550	825	1100	1375

\* Based on South African TB treatment guidelines (WHO 2008)

The formulation was a fixed dose combination (Rifafour<sup>®</sup>, Aventis Pharma, Johannesburg, South Africa) with each tablet containing 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide and 275 mg of ethambutol. Thus each patient received the appropriate number

of tablets for their weight. The above table constitutes the ‘intensive phase’ of TB treatment and the regimen is administered over a 2 month period. Thereafter, pyrazinamide and ethambutol are discontinued whilst rifampicin and isoniazid are administered for a further 4 months. Of the 154 patients, 78 were recruited into the pharmacokinetic sub-study.

The main conclusion from the parent study was that there is no difference between the vitamin A plus zinc compared with placebo on study outcomes in this cohort of patients which was followed over a two month period (Visser *et al.* 2010). Further details of this study can be found in the reference, but study procedures and a description of the patients are outlined below.

#### **4.1.1 Ethical considerations**

Both the parent study and the pharmacokinetic sub-study were approved by the Ethics and Research Committee of the University of Cape Town (Appendix 1). Further ethical approval was sought and obtained from the same ethics committee for genetic analysis related to drug transporter polymorphisms that may potentially influence rifampicin disposition (Appendix 1). Patients were only recruited into the study after written informed consent.

#### **4.1.2 The study site and study population**

The study population was comprised of adult patients with pulmonary TB being treated at the Delft Community Health Centre in Cape Town, South Africa. The patients were essentially outpatients, but would come to the centre from Monday to Friday to receive and swallow their antitubercular drugs under observation as part of the World Health Organization (WHO) Directly Observed Treatment Strategy (DOTS) program. Due to the clinic being closed on weekends, patients did not take their drugs on Saturdays and Sundays, although this practice

has now been discontinued and it is now current practice for the patients to take their drugs 7 days a week. The study population can be considered representative of the general hospital population in such a routine clinical setting due to generally broad inclusion criteria. In this study, the patients took their tablets after food to minimize the risk of possible gastrointestinal side effects (Visser *et al.* 2010).

#### **4.1.3 Eligibility criteria**

Patients were included in the study if they were at least 18 years old, but not more than 60 years of age. The patients also had to have 2 positive spontaneous sputum smear results, or 1 positive result and chest radiography indicative of TB. Patients were excluded if they had been previously treated for TB, if they were known or suspected to have MDR-TB, if there was clinical evidence for extra-pulmonary TB, or if the patients had renal or hepatic dysfunction based on renal and liver function tests. Patients were included in the study regardless of their HIV status or sex, although pregnant women were excluded due to potential teratogenicity of vitamin A.

#### **4.1.4 Patient demographics**

There were no significant differences between the characteristics of the patients in the sub-study when compared with patients not in the sub-study. Forty-seven (60%) of the 78 patients in the sub-study were males. Thirteen (17%) patients were HIV positive. Thirty-one (40%) patients were black Africans, whilst the remainder were admixed individuals. The admixture was mainly Caucasian-African in nature, although some patients may also have had an Asian component. Table 4.2 below shows the summary statistics for the continuous demographics.

Table 4.2: Patient demographics for pharmacokinetic study of first-line drugs

Characteristic	Median	2.5 <sup>th</sup> percentile	97.5 <sup>th</sup> percentile
Weight (kg)	53	42	74
Body Mass Index (kg/m <sup>2</sup> )	19	16	29
Height (m)	1.66	1.45	1.78
Age (y)	28	19	55

Body Mass Index (BMI) was calculated as the body weight in kg divided by the square of the patient's height in m.

#### 4.1.5 Pharmacokinetic sampling

Although patients were recruited from baseline (before they started treatment) blood samples for pharmacokinetic assessment were drawn about one month after patients had been on treatment. Thus the drug concentrations were at steady-state and the known rifampicin-mediated induction would be expected to be complete. The patients were sampled later on in the week rather than on Mondays, Tuesdays or Wednesdays since they would not have taken their weekend doses. Details of the 3 previous doses and times were accurately recorded.

Blood samples for drug plasma concentration determination were drawn quasi-randomly over a period of up to 7 hours after the dose. The samples were taken at convenient times but such that details of the entire drug concentration-time profiles could be captured when the data has been pooled together. Each patient generally had 4 samples drawn although 27 patients each had 8 samples drawn on 1 sampling occasion. Thirty-three patients were sampled on 2 separate occasions (about 1 month apart), whilst the remaining patients were only sampled once. Blood was immediately centrifuged after collection and the plasma was then stored on dry ice before later being transferred to a -80 °C freezer for long term storage.



#### **4.1.6 Drug plasma concentration determination**

Measurement of drug plasma concentrations was carried out using high performance liquid chromatography-tandem mass spectrometric methods by the University of Cape Town, Division of Clinical Pharmacology. Validated and published methods were used for rifampicin, isoniazid, pyrazinamide (McIlleron *et al.* 2007) and ethambutol (Jonsson *et al.* 2011). The drug concentrations were validated in the range of 0.1-30 mg/L for rifampicin, 0.1-15 mg/L for isoniazid, 0.2-70 mg/L for pyrazinamide and 0.1-10 mg/L for ethambutol. Inter- and intra-day coefficients of variation were less than 10% for all the drugs. Although the rifampicin assay was validated in the range 0.1-30 mg/L values down to 0.08 mg/L were measured from the laboratory and considered suitable for inclusion in the analysis. Values below 0.08 mg/L were not available and were indicated to be below the lower limit of quantification (LLOQ) for rifampicin. The data indicated to be below the LLOQ were not excluded, but were handled using Beal's M3 method where the likelihood of the data being below the LLOQ was estimated (Ahn *et al.*, 2008).

### **4.2 Assessment of pharmacodynamic activity of the first-line drug regimen**

#### **4.2.1 Collection and processing of sputum**

The 154 patients from the parent study each gave a morning sputum sample (collected overnight) at baseline and weekly thereafter for 8 weeks i.e. the entire intensive phase of treatment. The sputum samples were then decontaminated in accordance with the manufacturer's (Becton Dickinson<sup>TM</sup>) specifications as outlined in section 1.3.3. After decontamination, each processed specimen was inoculated into the BACTEC MGIT 960 system (Becton Dickinson<sup>TM</sup>, Sparks, MD) which was the automated liquid culture system comprised of Middlebrook 7H9 broth. The BACTEC MGIT 960 system is the culture

medium of choice in the South African National Laboratory Health Service (NHLS). The number of days to a positive MGIT test result was available automatically from the machine and was recorded in days. Absence of a positive test result after 42 days of incubation was considered a negative result; therefore the TTD data was censored at 42 days. If a positive result was found, a Ziehl Neelsen staining test for presence of acid-fast bacilli was undertaken to confirm that the positivity was due to growth of *M. Tuberculosis* in the MGIT culture. If acid-fast bacilli were absent, then this would be considered a contaminated specimen and would subsequently be a missing result in the dataset. Ten patients were omitted from subsequent analyses because they did not have a positive sputum culture result at baseline.

#### **4.2.2 Lung cavitation**

The presence of lung cavities at baseline was assessed by a pulmonologist experienced with the use of the Chest Radiograph Reading and Recording System (CRRS) (Den Boon *et al.* 2005). Ninety-seven (67%) of the patients had lung cavities visible on chest x-ray, 20 (14%) did not have lung cavities present, whilst 27 (19%) did not have x-ray data available.

### **4.3 Ofloxacin population pharmacokinetics and pharmacodynamics in patients with MDR-TB**

This was a study to describe the population pharmacokinetics of ofloxacin and thereafter assess the adequacy of ofloxacin drug exposure with respect to achieving the recommended pharmacokinetic-pharmacodynamic target, which was an AUC/MIC ratio of at least 100. Patients were on the standard intensive regimen for MDR-TB in South Africa which was comprised of weight based doses of kanamycin, pyrazinamide, ethionamide and terizidone, in

addition to 800 mg daily of ofloxacin. The study was conducted in Durban and in Cape Town (both of which are in South Africa) as will be outlined below.

#### **4.3.1 Ethical considerations**

Ethical approval for the Cape Town study was obtained from the University of Cape Town Research Ethics Committee and the South African Department of Health (appendix 2). Ethical approval for the study in Durban was obtained from the University of KwaZulu-Natal, the South African Department of Health, and the United States Centers for Disease Control and Prevention (CDC). All participants were recruited after written informed consent.

#### **4.3.2 Study sites and study population**

The study in Durban was conducted at King George V hospital and this was a pharmacokinetic sub-study of a study that was being conducted by the TB Trials Consortium (TBTC Study 30, funded by the CDC Division of Tuberculosis Elimination, <http://clinicaltrials.gov/show/NCT00664313>) whose title was “Safety and Tolerability of Low Dose Linezolid in MDR TB (LiMiT)”. Twenty-seven patients were recruited into the sub-study, 12 of whom received 600 mg of linezolid once daily whilst the rest received placebo. All patients were also on the standard MDR-TB treatment regimen described above.

A separate, unrelated study in Cape Town was designed to increase patient numbers in order to have a better description of the pharmacokinetics and pharmacodynamics of ofloxacin. Although it was a study independent from TBTC Study 30, the ofloxacin data was pooled together for the population modeling analysis. The Cape Town study was conducted at DP Marais Hospital in Retreat.

The 2 hospitals are referral hospitals whereby patients found to have MDR-TB are automatically referred to these centres from the primary health care system. The patients are quarantined in these hospitals and are not allowed to leave until they have negative sputum culture results.

#### **4.3.3 Eligibility criteria**

Patients with drug resistance to rifampicin and isoniazid as determined by drug susceptibility tests were recruited into the studies. The results were routinely available from patient clinical records. Study participants had to be at least 18 years old, with no upper limit for the age. Both males and females could participate, although pregnant women were excluded. However, DP Marais hospital was a male only TB hospital. Patients with severe anaemia as defined by a hematocrit less than 25%, or those with progressive renal disease based on renal function tests were also excluded from the study. For the patients in Durban, known intolerance to linezolid was an exclusion criterion, although this obviously did not apply to the Cape Town patients.

#### **4.3.4 Patient demographics**

Thirty-eight patients were recruited from Cape Town, whilst 27 were recruited from Durban, giving a total of 65 patients. Thirty-five (54%) of the 65 patients were HIV positive. Of these, 18 were from Cape Town and 17 from Durban. There were 13 females (20%) in the study, all of whom were from the Durban site. Table 4.3 describes the distribution of the continuous patient characteristics.

Table 4.3: Characteristics of MDR-TB patients

Characteristic	Median	2.5 <sup>th</sup> percentile	97.5 <sup>th</sup> percentile
Weight (kg)	55	39	80
Lean body weight (kg)	46	32	54
Height (m)	1.67	1.34	1.84
Age (y)	34	20	63
Body mass index (kg/m <sup>2</sup> )	19.3	13.6	36.4
Creatinine Clearance (mL/min)	109	69	159

Lean body weight (LBW) was calculated using the equations below (Janmahasatian *et al.* 2005):

$$LBW (male) = \frac{9.27 \times 10^3 \times Wt}{6.68 \times 10^3 + 216 \times BMI}$$

$$LBW (female) = \frac{9.27 \times 10^3 \times Wt}{8.78 \times 10^3 + 244 \times BMI}$$

where Wt is the patient's weight in kg.

Creatinine clearance was calculated from the Cockcroft-Gault equation:

$$Creatinine\ Clearance\ (mL/min) = \frac{(140 - age) * Wt * K}{sCr}$$

where K is a constant equal to 1.04 for women and 1.23 for men, and sCr is measured serum creatinine concentration in µmol/L.

#### 4.3.5 Durban pharmacokinetic sampling

Pharmacokinetic sampling took place at least 14 days after initiation of treatment thus all the patients were at steady state. The sampling schedule was 0, 1, 2, 4, 8, 11 and 24 h after dose administration i.e. a total of 7 samples per individual on one occasion. The dose was typically given in the morning before breakfast, after an overnight fast. After drawing each blood sample from the patients, the lithium heparin tubes were immediately placed on ice. The

tubes were then centrifuged and the plasma was separated and aliquoted into cryotubes within 30 minutes. The tubes containing the plasma were placed on ice, transported, and stored at -70°C within 4 h of collection.

#### **4.3.6 Cape Town pharmacokinetic sampling**

Pharmacokinetic sampling was also undertaken at least 2 weeks after initiation of treatment. Five blood samples were drawn from each patient on one occasion, with an approximate sampling schedule of 0.5, 3.5, 5.5, 7.5 and 12 h after the dose. The dose was administered after breakfast which was comprised of oatmeal porridge, bread and a cup of tea. The sampling schedule was based on a skeleton of 3 sampling windows which were 0.4 – 0.8, 3.0 – 4.1 and 9.4 – 12.9 h after the dose. The software program WinPOPT<sup>®</sup> Version 1.2.1 (University of Otago, Otago, New Zealand, <http://winpopt.com/>) was used to obtain these sampling windows based on optimal design theory. WinPOPT<sup>®</sup> works by computation of the Fisher Information Matrix given a model and a set of study design parameters. The input population pharmacokinetic model implemented in WinPOPT<sup>®</sup> was from literature and had typical values (% variability) of clearance, volume of distribution and the absorption rate constant of 8.4 L/h/70kg (31%), 89.6 L/70kg (25%) and 1.03 h<sup>-1</sup> (124%) respectively (Stambaugh *et al.* 2002).

For the pharmacokinetic sampling, patient blood samples were drawn into lithium heparin tubes and immediately placed on ice after collection. The samples were then centrifuged within 10 minutes of collection and the aliquoted plasma was placed on dry ice before being transferred all at once to a -80 °C freezer at the end of the day.

#### **4.3.7 Drug plasma concentration determination**

Drug plasma concentrations were determined by the University of Cape Town, Division of Clinical Pharmacology. A validated high-performance liquid chromatography assay with tandem mass spectroscopy detection method was used (Meredith *et al.* 2012). The assay was validated over the concentration range of 0.078 mg/L to 20 mg/L. The percentage recovery for ofloxacin was greater than 70% and reproducible at low, medium and high concentrations (0.25, 8.0 and 16 mg/L). The coefficient of variation during analysis of the study samples ranged from 4.4% to 6.0% and the accuracy from 94.0% and 102.9% for high, medium and low quality controls, showing that the method had good reproducibility (Meredith *et al.* 2012).

#### **4.3.8 Determination of the ofloxacin MICs in patient sputum isolates**

The MICs were kindly determined by the University of KwaZulu-Natal Department of Infection Prevention and Control in Durban. Therefore the Cape Town tubes were couriered to the laboratory in Durban. The MIC determination was based on the agar dilution method using two-fold dilutions from an initial concentration of 8.0 mg/L down to 0.03 mg/L. Serial ofloxacin concentrations as well as a drug-free control were incorporated into Middlebrook 7H10 agar. A 1.0 McFarland standard of each *M. tuberculosis* isolate from the patients was diluted to 10<sup>-4</sup> before inoculation onto the drug-free control and drug-containing quadrants. A sensitive H37Rv and a resistant A169 *M. tuberculosis* control were set up with each batch of MICs. The inoculated agar plates were allowed to air dry in a bio-safety cabinet and then packed into carbon dioxide permeable plastic bags. The bags were sealed and incubated at 37 °C and the plates were read after 21 days. The lowest ofloxacin concentration on the agar plate that did not have growth was recorded as the MIC. MIC results were available for all 38

Cape Town patients, and 22 of the 27 Durban patients. In both cases, these were the baseline sputum sample MICs, before treatment commenced.

#### **4.4 Pharmacometric analyses**

The general approach to the data analysis is described here, although more specific information can be found in the relevant methods sections of chapters to follow. Apart from the TBTC Study 30 in Durban, all the other drug concentration profiles were sparse in nature. In addition, the sampling times were not the same for all patients for the pharmacokinetic study of the first-line drugs. The sampling times for TBTC Study 30 were different from those for the Cape Town study in patients with MDR-TB although the data was pooled together for analysis. All of the above reasons provide justification for the use of population modeling methods rather than non-compartmental analyses. NLME modeling implemented in the NONMEM<sup>®</sup> software program (Icon Development Solutions, Verona, PA) version 7 was the primary data analysis method. The program was operated on a cluster of LINUX machines using an Intel Fortran Compiler. The modeling runs were executed using Perl-Speaks-NONMEM<sup>®</sup> (PsN, <http://psn.sourceforge.net/>). Various versions of the R program (R\_Development\_Core\_Team 2008) and the xpose package in R (<http://xpose.sourceforge.net/>) were used for model diagnostics and plotting.

##### **4.4.1 Model building and evaluation**

The estimation algorithm used most often was the first order conditional estimation method (FOCE) with epsilon-eta ( $\epsilon$ - $\eta$ ) interaction. Model parameters were generally added one at a time starting from the base model. A decrease in the goodness-of-fit criterion, the objective function value (OFV) of at least 3.84 points ( $p < 0.05$ ) when comparing 2 hierarchical models



was regarded as statistically significant for the addition of a single model parameter. Covariates were tested one at a time in a stepwise forward inclusion manner at a 5% significance level. A backward deletion step at the 1% significance level would be performed after inclusion of all significant covariates. Clinical relevance and biological plausibility were also taken into consideration as part of the model building process. Continuous covariates were generally centred around the median value except in the case of body weight which was introduced into the models using allometric scaling and centred around a value of 70 kg (Anderson and Holford 2008). In most cases, population variability in model parameters was introduced using a log-normal distribution i.e.:

$$PAR_i = TV(PAR) \times EXP^{\eta_i^{PAR}}$$

where  $PAR_i$  is the estimated value of the parameter  $PAR$  in individual  $i$ ,  $TV(PAR)$  is the typical population value of the parameter, and  $\eta_i^{PAR}$  is a measure of the individual  $i$ 's deviation from the typical population value.

$\eta_i^{PAR}$  is drawn from a variance  $\Omega$  which is estimated by NONMEM. The log-normal distribution of the random effects (population variability) is more representative of biological systems especially for pharmacokinetic model parameters (generally right-skewed) and also has the advantage that a negative parameter cannot be predicted. However, this is not always the case and in some cases model parameters may need to be negative or positive e.g. some parts of disease progression models and as such the above parameterisation would not be used.

As aforementioned, some patients had pharmacokinetic sampling done on 2 separate occasions about 1 month apart from each other. They thus had 2 pharmacokinetic profiles which were labelled as OCC=1 and OCC=2 in the dataset. In NONMEM, within subject

variability (WSV) was modelled using the code below, which uses Clearance (CL) as an example.

\$PK

IF(OCC.EQ.1) WSV\_CL = ETA(X) ; occasion 1 ETA

IF(OCC.EQ.2) WSV\_CL = ETA(Y) ; occasion 2 ETA

BSV\_CL = ETA(Z) ; between subject variability ETA

CL = TVCL\*EXP(BSV\_CL + WSV\_CL)

\$OMEGA

0.1 ; initial estimate for variance of ETA(X), occasion 1

SAME ; estimate for variance of ETA(Y) for occasion 2 constrained to be the same as that for occasion 1

0.1 ; initial estimate for between subject variability in CL

The OFV, goodness-of-fit plots and visual predictive checks (VPCs) were used as methods of model evaluation. VPCs were performed by using PsN to simulate 1000 datasets based on the final model. The 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of the observations were then visually compared with the corresponding 95% confidence intervals for the simulated data, thus giving a 90% prediction interval. The goodness-of-fit plots were comprised of plots of the observed data vs. population predictions (structural model only), plots of the observed data vs. individual predictions (structural and variability models), plots of the individual weighted residuals vs. individual predictions, and plots of the weighted residuals against time. Stability of parameter estimates was also used to guide decisions relating to the inclusion of model parameters. Where possible, measures of parameter uncertainty were obtained from the covariance step in NONMEM<sup>®</sup> which outputs the relative standard errors. In cases of failure to obtain the

covariance matrix, measures of parameter uncertainty were obtained through using PsN to run a bootstrap of the final model. The number of bootstrap replicates performed depended upon the run times of the model, with no fewer than 200 replicates being performed for each case.

For pharmacokinetic data that were below the LLOQ the Beal M3 method was used (Ahn *et al.* 2008). For this method, whenever a measurement in the dataset was recorded as being below the LLOQ, the likelihood that the model-predicted drug concentration was less than the LLOQ was estimated i.e. the likelihood that the concentration was somewhere between minus infinity and the LLOQ. For this likelihood-based method, the Laplacian estimation method was used instead of FOCE.

When required, the time integral of the drug concentrations ( $AUC_{0-24}$ ) was obtained by creating an additional compartment in NONMEM where the amount of drug in the central compartment, divided by the volume of distribution, would accumulate into from 0-24 h after the dose. The  $C_{max}$  would be obtained by using NONMEM to determine the maximum concentration of the predicted drug concentration-time profile.

## **4.5 Declaration of work**

### **4.5.1 Population pharmacokinetics of first-line drugs**

Recruitment of patients with ordinary TB, pharmacokinetic sampling, follow up and all data collection for the work described in section 4.1 was carried out by a study team and not the candidate. The candidate was involved with data processing and cleaning. All the genotyping work described in chapter 5.2.2 was carried out by the candidate. The haplotyping analysis

using the Haploview software was carried out by the University of Liverpool Department of Pharmacology and Therapeutics. All drug plasma concentration determination was carried out by the University of Cape Town Division of Clinical Pharmacology. All the data analysis, modeling and simulation were performed by the candidate.

#### **4.5.2 Ofloxacin Pharmacokinetics and Pharmacodynamics in Patients With MDR-TB**

As aforementioned, TBTC Study 30 was responsible for the conduct of the study in Durban. For the study in Cape Town, the candidate was responsible for designing, implementation and the conduct of the entire study, the optimal design of the pharmacokinetic sampling points, recruitment and follow up of patients, data collection, data analysis, modeling and simulation. The MICs for both patient cohorts were determined by the University of KwaZulu-Natal Department of Infection Prevention and Control. Ofloxacin plasma concentration determination was carried out by the University of Cape Town Division of Clinical Pharmacology.

#### **4.5.3 Clinical Trial Simulation**

All the work described in Chapter 12 was carried out by the candidate.

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The following chapters describe the work carried out to determine the population pharmacokinetics of the individual TB drugs in South African patients, the pharmacodynamics based on days to culture in liquid media, the effect of the pharmacokinetics on the pharmacodynamics, clinical trial simulation to optimize antitubercular drug studies, and the overall discussion and conclusions.

## **5. THE *SLCO1B1* rs4149032 POLYMORPHISM IS HIGHLY PREVALENT IN SOUTH AFRICANS AND IS ASSOCIATED WITH REDUCED RIFAMPICIN CONCENTRATIONS: CLINICAL AND DOSING IMPLICATIONS**

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### **5.1 Introduction**

The development of resistance to rifamycins and their bactericidal effects are related to rifampicin concentrations (Jayaram *et al.* 2003; Weiner *et al.* 2005; Diacon *et al.* 2007; Gumbo *et al.* 2007), with some authors recommending a minimum C<sub>max</sub> of 8 mg/L (Peloquin 1996; Perlman *et al.* 2005). There is high intersubject variability of rifampicin plasma concentrations in patients with TB, some of which is explained by HIV infection, sex, formulation and weight (Gurumurthy *et al.* 2004; Perlman *et al.* 2005; McIlleron *et al.* 2006; Wilkins *et al.* 2008). Rifampicin concentrations are lower in patients from sub-Saharan Africa (Weiner *et al.* 2010), which may contribute to the lower sputum culture conversion at two months in African patients that has been recently reported in two multicentre phase 2 trials of moxifloxacin in rifampicin-based antitubercular regimens (Burman *et al.* 2006; Dorman *et al.* 2009). Population differences in drug concentrations may be partly the result of differences in genes encoding drug metabolising enzymes or transporters. Little is known about pharmacogenetic determinants of rifampicin exposure. Since about 50% of a rifampicin dose is excreted via the bile unchanged (Sanofi-Aventis 2007), polymorphisms of drug transporters and/or their transcriptional regulators may influence rifampicin pharmacokinetics.

The objectives of this study were: 1) to determine the effect of polymorphisms of *ABCB1*, *SLCO1B1*, and the transcriptional regulators the *PXR* and the *CAR* on rifampicin

pharmacokinetics, 2) to determine the influence of rifampicin drug exposure as part of a multidrug regimen on treatment outcome and 3) to evaluate via simulation, the utility of dose adjustment based on *SLCO1B1* genotype.

## **5.2 Methods**

### **5.2.1 Study participants**

The group of study participants were being treated for ordinary TB as outlined in the methods section 4.1. An additional blood sample was collected for genetic analyses after separate written consent was obtained from 60 of the 78 patients in the pharmacokinetic substudy.

### **5.2.2 Genotyping**

Genomic DNA was extracted from the blood samples using the Qiagen Inc. QIAamp DNA blood mini kit (Hilden, Germany). Real time PCR using fluorescent probes for allelic discrimination was used for genotyping. Primers and probes were sourced from Applied Biosystems Inc. (Warrington, United Kingdom). Absolute quantitative PCR mix was obtained from ThermoFisherScientific (Loughborough, United Kingdom). The PCR conditions for most SNPs were: initial denaturation at 95 °C for 15 minutes, followed by 40-50 cycles of denaturation at 95 °C for 15 seconds then annealing and extension at 60 °C for 60 seconds, with a plate read after each cycle. For *SLCO1B1 T521C* the denaturation temperature was 92 °C. Each PCR reaction contained 2 µL of genomic DNA, 12.5 µL of absolute quantitative PCR mix (2X), 1.25 µL primer mix (20X) and 1.25 µL of probe mix (20X), made up to 25 µL with water. The assays were run on a Chromo4 Real Time PCR Detection System (Bio-Rad Life Sciences, United Kingdom). The following SNPs were genotyped for, based on previous reports of allele frequency and functional significance:

*ABCB1* (*C3435T*, *G2677T*, *C1236T* and rs3842), *SLCO1B1* (*T521C*, *C463A* [rs11045819] and rs4149032), *PXR* (*C63396T* and *T44477C*) and *CAR* (rs2307424). All genotyping assays were performed in duplicate and genotype was only assigned when the two separate assays were in agreement. Haplotype analysis of the *SLCO1B1* gene was performed using the Haploview software (Barrett *et al.* 2005).

### 5.2.3 Drug plasma concentration determination

Blood samples were centrifuged to obtain plasma, which was stored at -80 °C until analysis. Rifampicin concentrations were measured using high performance liquid chromatography with tandem-mass spectroscopy (McIlleron *et al.* 2007) as summarized in section 4.1.6.

### 5.2.4 Pharmacokinetic analyses

Pharmacokinetic data from 75 patients were available yielding a total of 607 observations. Three patients did not have measurable rifampicin concentrations throughout the sampling schedule and were excluded from the analysis. Twelve percent of the observed data was below the LLOQ (0.08 mg/L) and was entirely in the absorption phase (i.e less than 2 hours after the dose had been taken). Beal's M3 method (Ahn *et al.* 2008) was used to handle data below the LLOQ. A transit absorption compartment model was used to account for variability in the absorption delay (Savic *et al.* 2007; Wilkins *et al.* 2008). First order elimination from a one compartment model best described the remaining structural model.

The effects of several covariates on model parameters were investigated one at a time in a stepwise fashion. Covariates investigated included study arm (vitamin A and zinc vs. placebo) body weight, age, HIV status, sex and the daily dose of rifampicin. The covariates for the pharmacogenetic analyses included *ABCB1* (*C3435T*, *G2677T*, *C1236T* and rs3842),

*SLCO1B1* (*T521C*, *C463A* and *rs4149032*), *PXR* (*C63396T* and *T44477C*) and *CAR* (*rs2307424*) polymorphisms. Only 57 of the 75 patients with pharmacokinetic data had DNA available for genotyping. For the remaining 18 patients, a mixture model was used to determine the probability of an individual belonging to a particular genotypic subpopulation.

The final model was used to predict individual  $C_{max}$ 's for each patient. Sixty nine of the patients had week 8 sputum MGIT culture results as outlined in section 4.1. A 2 X 2 contingency table was constructed to determine whether a  $C_{max}$  above or below a threshold of 8 mg/L was associated with the 8 week treatment outcome using a Chi-square test. The final model was also used to obtain the AUC for each individual by creating an additional compartment where drug accumulated from 0-24 hours after the dose. A Mann-Whitney U test was used to investigate the difference in AUCs between carriers and non-carriers of the *rs4149032* polymorphism.

### **5.2.5 Dosing simulations**

Using the final model, deterministic simulations were carried out to obtain plasma concentration-time profiles for patients weighing 46 kg and 62.5 kg, which represent the weights in the middle of the standard dosing weight bands (Health and Medical Publishing Group 2010) in order to visualize the effect of genetic polymorphism on the pharmacokinetic profile. The patients would receive 450 mg and 600 mg rifampicin doses once daily respectively. Thereafter, stochastic simulations to obtain drug concentration-time profiles for all patients based on the final model were carried out to investigate the applicability of dose adjustment based on genotype. These were Monte Carlo simulations in 1000 patients based upon the covariate distribution of the current dataset. The percentages of simulated  $C_{max}$



above and below 8 mg/L were then calculated for wild-type individuals, and for patients who were hetero- or homozygous for the polymorphism, using the standard weight based dosing. This process was then repeated but with carriers of the polymorphism receiving an additional 150 mg dose to compensate for decreased bioavailability.

### 5.3 Results

The *SLCO1B1* rs4149032 polymorphism existed at an overall frequency of 0.70 in our study population. The frequency in males was 0.69 whilst in females it was 0.76. Frequency in Black Africans was 0.93, whilst in the mixed race group it was 0.59. Thirty one individuals were homozygous (52%) for the reported variant allele, whilst 22 (37%) were heterozygotes, and 7 (12%) were homozygotes for the common (wild-type) allele. The remaining allele frequencies for the various SNPs that were genotyped are shown in the table below.

Table 5.1: Allele Frequencies

Allele	Frequency
<i>ABCB1</i> C3435T	0.26
<i>ABCB1</i> G2677T	0.19
<i>ABCB1</i> C1236T	0.26
<i>ABCB1</i> rs3842	0.21
<i>PXR</i> C63396T	0.27
<i>PXR</i> T44477C	0.27
<i>CAR</i> rs2307424	0.11
<i>SLCO1B1</i> T521C	0.05
<i>SLCO1B1</i> rs4149032	0.70
<i>SLCO1B1</i> C463A	0.04

There were at most three homozygous individuals for any of these polymorphisms (except *SLCO1B1* rs4149032) in the study population, therefore carrier/non-carrier assignments were used for subsequent analyses. Only 3 individuals that were carriers of the *SLCO1B1* 521C allele were observed and this SNP was therefore not included in any statistical analysis. Similarly, only 4 individuals were carriers of the *SLCO1B1* 463A allele. All polymorphisms were in Hardy-Weinberg equilibrium. The linkage disequilibrium (LD) plot of the 3 *SLCO1B1* SNPs from Haploview is shown in the figure below. The shading within the boxes indicates extent of LD between respective single nucleotide polymorphisms, with white indicating weaker LD than grey.

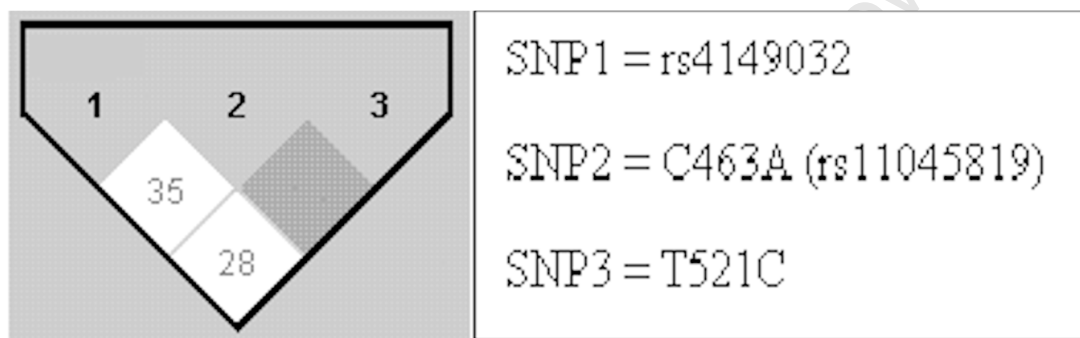


Figure 5.1: LD plots for the assessed SNPs in *SLCO1B1*.

The inferred haplotypes were then tested to determine their effect on rifampicin pharmacokinetics. However, similar results were obtained with results obtained for rs4149032 alone; hence rs4149032 was used for the subsequent analyses.

Table 5.2 below outlines the model building steps taken to reach the final model. Population parameter estimates from the final model and their relative standard errors (RSE) from the NONMEM covariance step are shown in table 5.3. The final control stream that produced these estimates can be found in appendix 3.1.

Table 5.2: Summary table of rifampicin population model development

Model	Description*	OFV	Comment
1	One compartment, first order absorption, first order elimination. PPV on CL, V, KA, F	1926	
2	Add lag time to model 1	1800	Adding variability to the lag time resulted in model instability and sometimes higher OFV than 1800 hence it was not included.
3	Transit compartment absorption model instead of lag time. Variability on CL, V, F, KA	1707	
4	Transit compartment absorption model instead of lag time. Variability on MTT instead of KA	1472	Significant improvement to lag model.
5	Saturable elimination, Vmax and Km instead of CL	1499	Worse fit, hence development of first order clearance model was continued
6	Add WSV CL to model 4	1427	
7	Add WSV for F to model 6	1413	
8	Add WSV MTT to model 7	1336	
9	Add WSV V to model 8	1206	BSV V went to zero and was subsequently removed
10	Add covariance between BSV CL and MTT	1174	Covariance between WSV CL and MTT did not change OFV
11	Add covariance between WSV MTT and V	1168	
12	Test enterohepatic circulation model	1153	4 additional parameters included i.e. start time of gall bladder emptying, duration of emptying, rate constant of flow from central compartment to gall bladder (estimated to be 0.0049/h), and flow from gall bladder to absorption compartment. Not included in final model due to small amount of drug entering gall bladder compartment and small change in OFV for 4 parameters
13	Allometric scaling of weight on CL and V to model 11	1162	
14	Add sex covariate on MTT to model 13	1157	
15	Add sex covariate on V to model 13	1155	Same magnitude of estimated effect for model 14 and 15, hence subsequent model had 1 theta for the 2 parameters and same OFV
16	Add effect of higher dose shortening MTT	1151	Retained for clinical significance of saturation of efflux pumps in

			intestinal lumen
17	Add SLCO1B1 rs4149032 genotype on CL to model 16	1137	
18	Add SLCO1B1 rs4149032 genotype on V to model 16	1127	Similar magnitude of effect as that found on CL, hence need to test on F
19	Add SLCO1B1 rs4149032 genotype on F to model 16	1130	Main finding and final model. Other polymorphisms were not significant on F or other model parameters. Removal of variability in F did not result in OFV change and it was excluded from the final model.

\* PPV – Population variability, CL – Clearance, V – Volume, KA – first order absorption rate constant, MTT – Mean absorption transit time, BSV – Between subject variability, WSV – Within subject variability

Table 5.3: Parameter estimates from final rifampicin population pharmacokinetic model

Parameter*	Estimate(%RSE)
CL/F L/h/70kg	11 (4.7)
V/F L/70kg	46 (7.3)
KA 1/h	1.0 (11)
MTT h	1.5 (8.7)
NN	19 (fixed)
Additive error mg/L	0.03 (0.7)
Proportional error	0.30 (3.5)
Effect of female sex on V/F %	-27 (23)
Effect of female sex on MTT %	-27 (23)
Effect of <i>SLCO1B1</i> rs41490932 on F in heterozygotes %	-22 (11)
Effect of <i>SLCO1B1</i> rs41490932 on F in variant homozygotes%	-28 (21)
Effect of dose on MTT %	-28 (25)
Mixing probability – Proportion of patients with F=1 (no rs41490932 polymorphism)	0.07 (192)
BSV of CL %	23 (38)
BSV of MTT %	71 (21)
Correlation between BSV of CL and MTT	0.95 (22)
WSV of CL %	42 (12)
WSV of V %	30 (14)

WSV of MTT %	60 (10)
Correlation between WSV of V and MTT	-0.42 (14)

\* RSE – Relative standard error, NN – number of transit compartments

Based upon a VPC (figure 5.2), the pharmacokinetic model was found to predict the data acceptably, particularly in the absorption phase which contained many observations. Only a fraction of the dosing interval was captured which may explain why the model predictions are slightly lower than the observations at later times. The model was also able to accurately predict the proportion of the observed data that was below the LLOQ throughout the pharmacokinetic sampling interval. The VPC is truncated at the LLOQ for both predictions and observations. The lower panel is a VPC for the censored data whereby the blue shaded area is the 95% confidence interval of the simulated proportion of data below the LLOQ concentration of 0.08 mg/L, whilst the open blue circles represent the proportion of the observed data that was below the LLOQ.

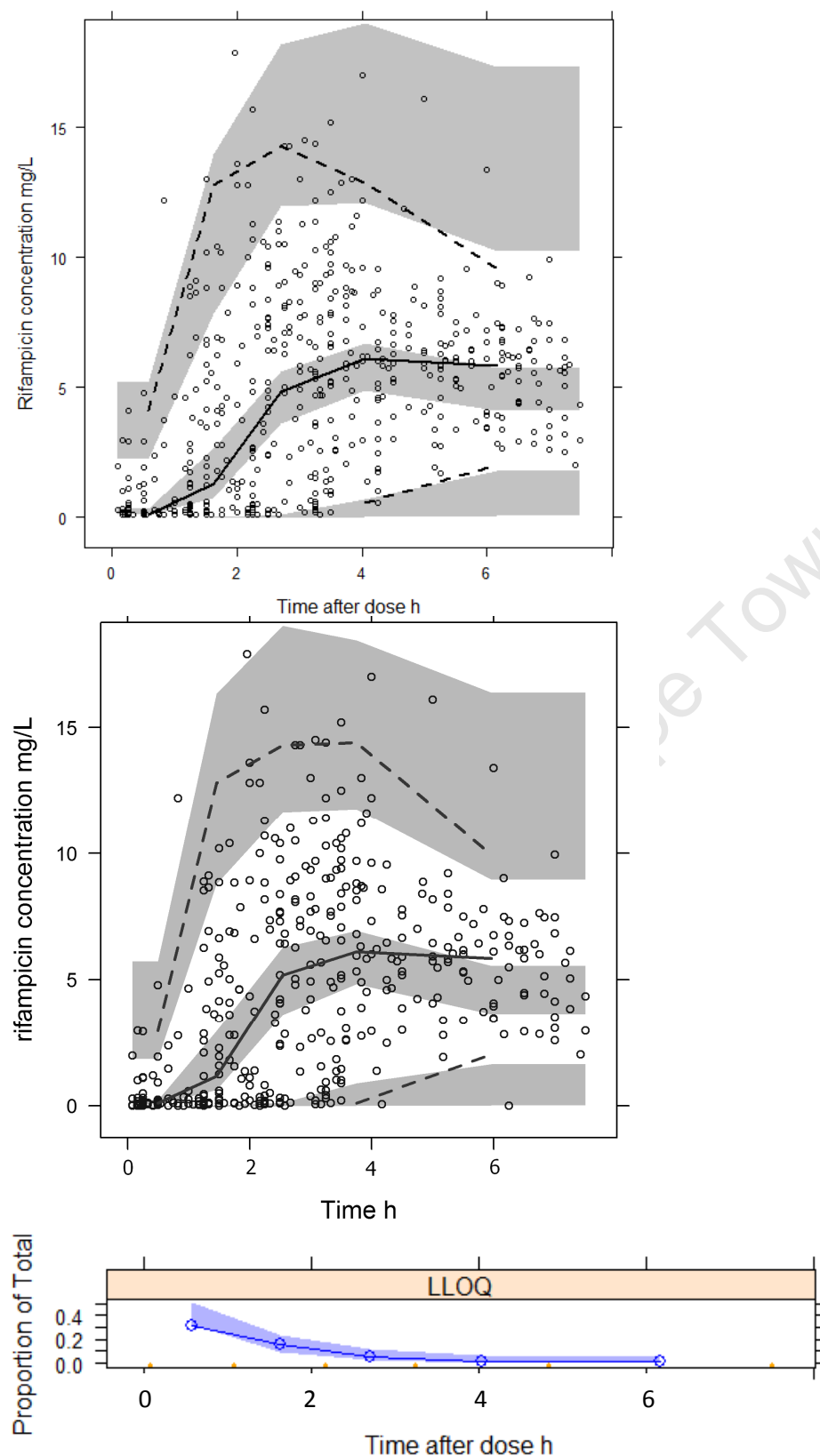


Figure 5.2: Visual predictive check of models. Top: Base model with no covariates (model 11). Middle: Final model with covariates (model 19). Lower: LLOQ VPC of final model. The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower

dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

For a clearer picture of the low concentrations, a log-transformed visual predictive check of the same model is shown below.

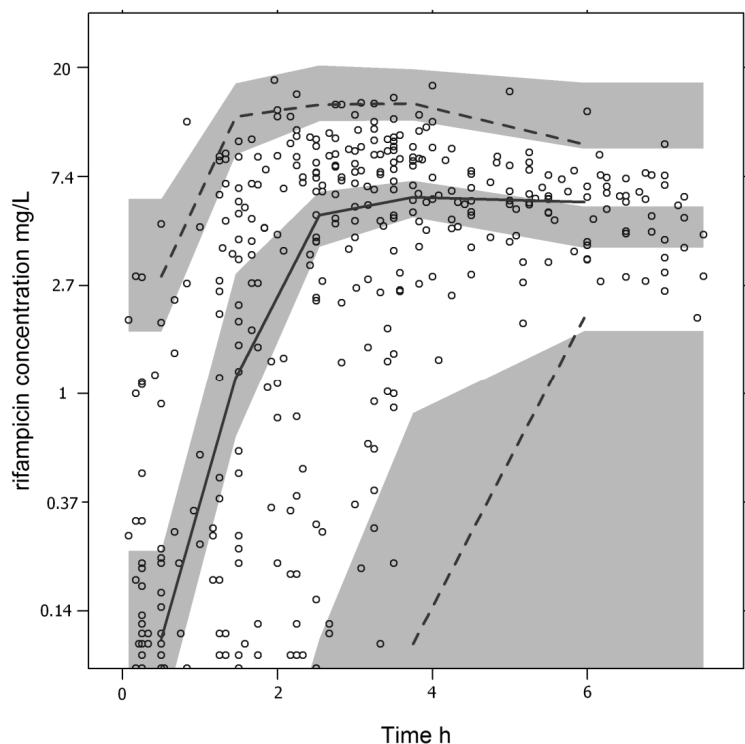


Figure 5.3: VPC on log scale for the final rifampicin model

The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

Clearance (CL/F) and apparent volume of distribution (V/F) were allometrically scaled for weight (Holford, 1996) and this resulted in a drop in the objective function value of 6 points when compared to a model without weight. The association of *SLCO1B1* rs4149032 with differences in oral bioavailability relative to the fixed nominal population value of 1 was incorporated in a categorical manner. Heterozygotes had 22% lower bioavailability and homozygotes had 28% lower bioavailability compared with homozygotes for the *SLCO1B1* rs4149032 allele. Inclusion of this effect also resulted in a 21% decrease in the between

subject variability for CL/F. The *SLCO1B1* rs41490932 polymorphism was associated with an apparent increase in the mean transit time of 9% for heterozygotes and 18% for homozygotes. However, as this effect was not statistically significant, it was not included in the final model. A mixture model with three sub-populations was investigated as an alternative way of identifying phenotypic polymorphisms however this approach did not improve the OFV compared with the genetic polymorphism based method.

*ABCB1* C3435T, C1236T, *CAR* rs23047424, *PXR* T44477C and C63396T polymorphisms did not have a statistically significant association with CL/F, V/F or relative bioavailability. The *ABCB1* G2677T polymorphism resulted in a 19% increase in CL/F and a 19% increase in mean transit time. Although inclusion of the *ABCB1* G2677T polymorphism in the model decreased the OFV by 4 points ( $P < 0.05$ ), as the VPC was unchanged it was not included in the final model for reasons of parsimony.

Women had a 27% lower V/F compared to men. At the same time, women had 27% longer mean transit time indicating that women have a longer absorption delay than men. In contrast, both men and women given higher doses (600 or 750 mg of rifampicin daily) were found to have a 28% shorter absorption delay compared to those given 450 mg daily. Individuals with a higher dose also had CL/F reduced by 18% but this was not statistically significant and was not included in the final model. Assignment of micronutrient study arm (vitamin A and zinc vs placebo) had no significant effect on rifampicin pharmacokinetics.

In order to evaluate if differences in the combined genotype associated effects on bioavailability were also associated with differences in overall drug exposure, AUCs from 0-24 h after dose administration ( $AUC_{0-24}$ ) were generated from the final model for each



individual. After stratification by *SLCO1B1* rs4149032 genotype there was no statistically significant difference in the  $AUC_{0-24}$  between heterozygotes and homozygotes. However a Mann-Whitney U test showed the median  $AUC_{0-24}$  of 45 mg.h/L in individuals with the polymorphism to be significantly lower than the median of 56 mg.h/L found in those without the polymorphism ( $p < 0.05$ ).

Using a reference minimum  $C_{max}$  of 8 mg/L (Peloquin 2002; Perlman *et al.* 2005), a typical individual without the polymorphism was predicted to achieve adequate plasma concentrations, whilst a typical individual with the polymorphism was under-dosed.

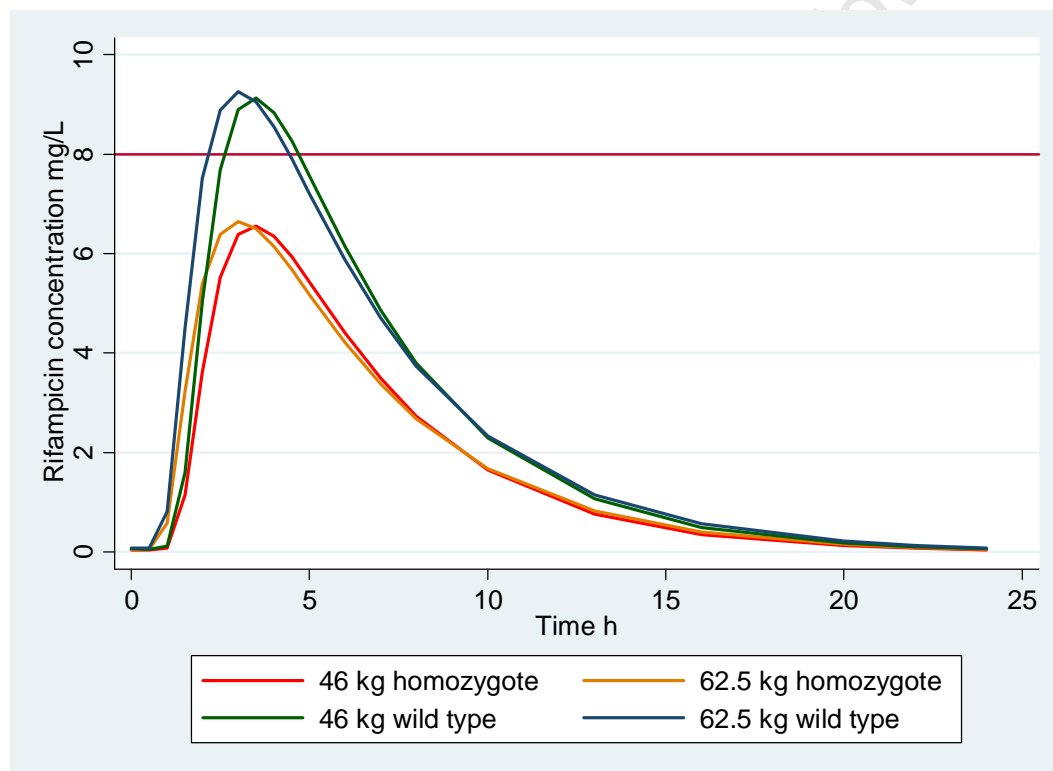


Figure 5.4 The predicted rifampicin plasma concentration time profiles for typical individuals weighing 46 and 62.5 kg, homozygous for the common allele and minor alleles respectively with standard weight based dosing.

As the bioavailability was found to be 28% lower in homozygous individuals with the polymorphism, the effect of a 150 mg dose increase for both homo and heterozygous *SLCO1B1* rs4149032 individuals was simulated as there was no statistically significant

difference in AUC between them. Thus, 46 kg carriers of the variant allele would receive 600 mg instead of the currently recommended 450 mg, and 62.5 kg carriers of the variant allele would receive 750 mg once daily resulting in the concentration-time profiles in figure 5.5.

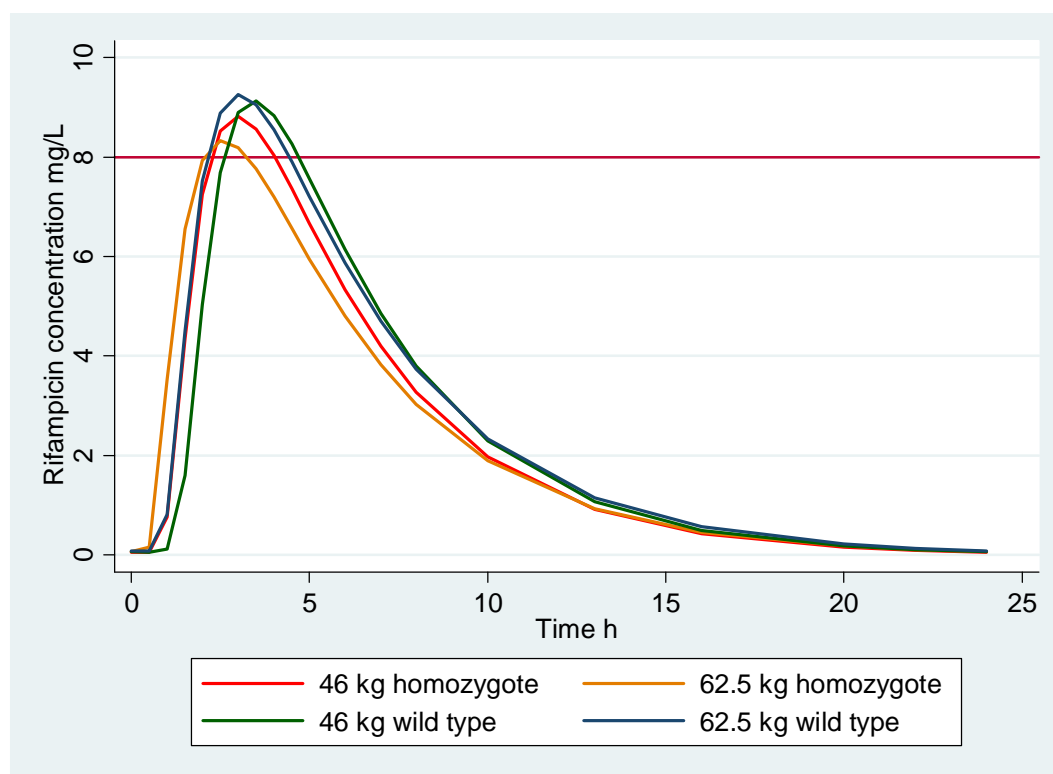


Figure 5.5: The predicted rifampicin plasma concentration time profiles for typical individuals weighing 46 and 62.5 kg, homozygous for the common allele and minor alleles respectively after an additional 150 mg for those homozygous for the common allele.

The deterministic simulations of a single typical individual above did not take into account population variability or residual variability. Therefore, Monte Carlo simulations were carried out based on the actual dataset using either the currently recommended doses or a genotype-based dose whereby *SLCO1B1* rs41490932 carriers received an additional 150 mg of rifampicin daily. The genotype-based doses resulted in a predicted reduction in the proportion of carriers with a C<sub>max</sub> of <8 mg/L from 65% to 32%. Standard dosing resulted in 30% of patients who did not carry the polymorphism having a C<sub>max</sub> of <8 mg/L. The percentage of patients with a C<sub>max</sub> of <8 mg/L in the entire study population was reduced from 62% to

32% after genotype-based dose adjustment. Figure 5.6 shows box and whisker plots for the range of C<sub>max</sub>'s for carriers based on simulations before and after dose adjustment compared with non-carriers of the *SLCO1B1* rs4149032 polymorphism.

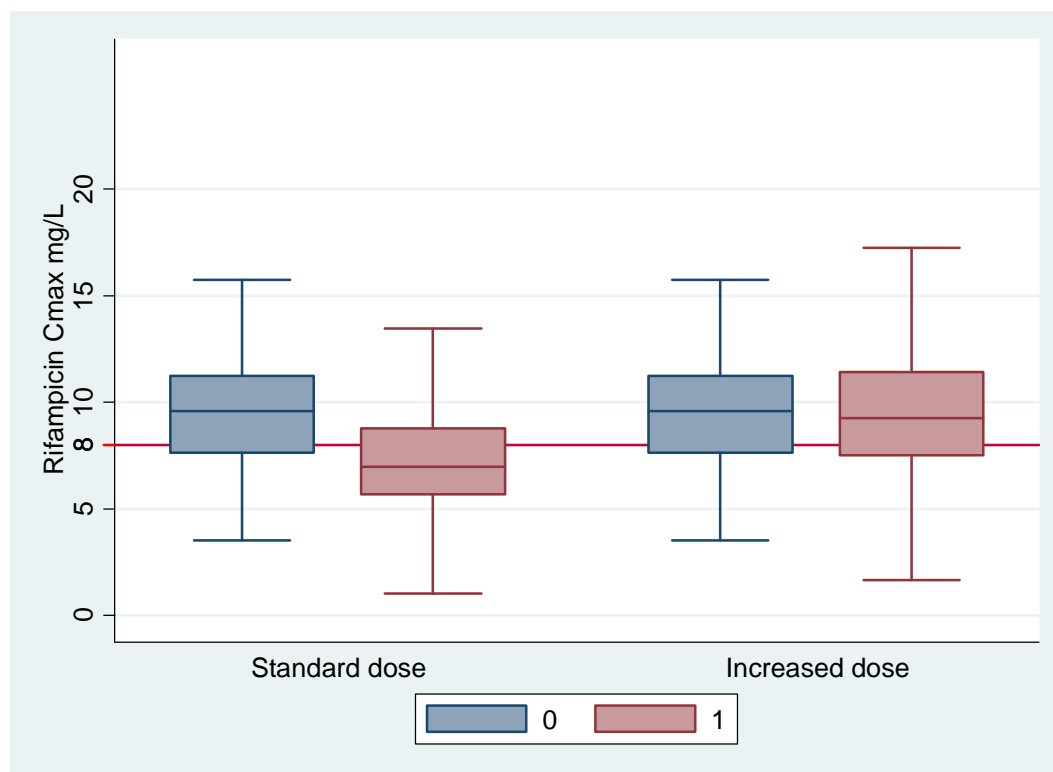


Figure 5.6: Box (interquartile range) and whisker (95 percentiles) plot of simulated variation in rifampicin C<sub>max</sub> based on the final model. Simulations were performed using the currently recommended doses (standard dose), and a genotype-based dose (Increased dose) whereby *SLCO1B1* rs4149032 carriers received an additional 150 mg of rifampicin daily. In the figure, 0 represents wild-type individuals, whilst 1 represents carriers of the polymorphism.

Histograms of the AUC<sub>0-24</sub> as well as for the C<sub>max</sub> for the patients in the dataset are shown in figure 5.7 below. The median (interquartile range) for the AUC<sub>0-24</sub> and C<sub>max</sub> were 46 (37-61) mg.h/L and 7.5 (6.1-9.2) mg/L, respectively.

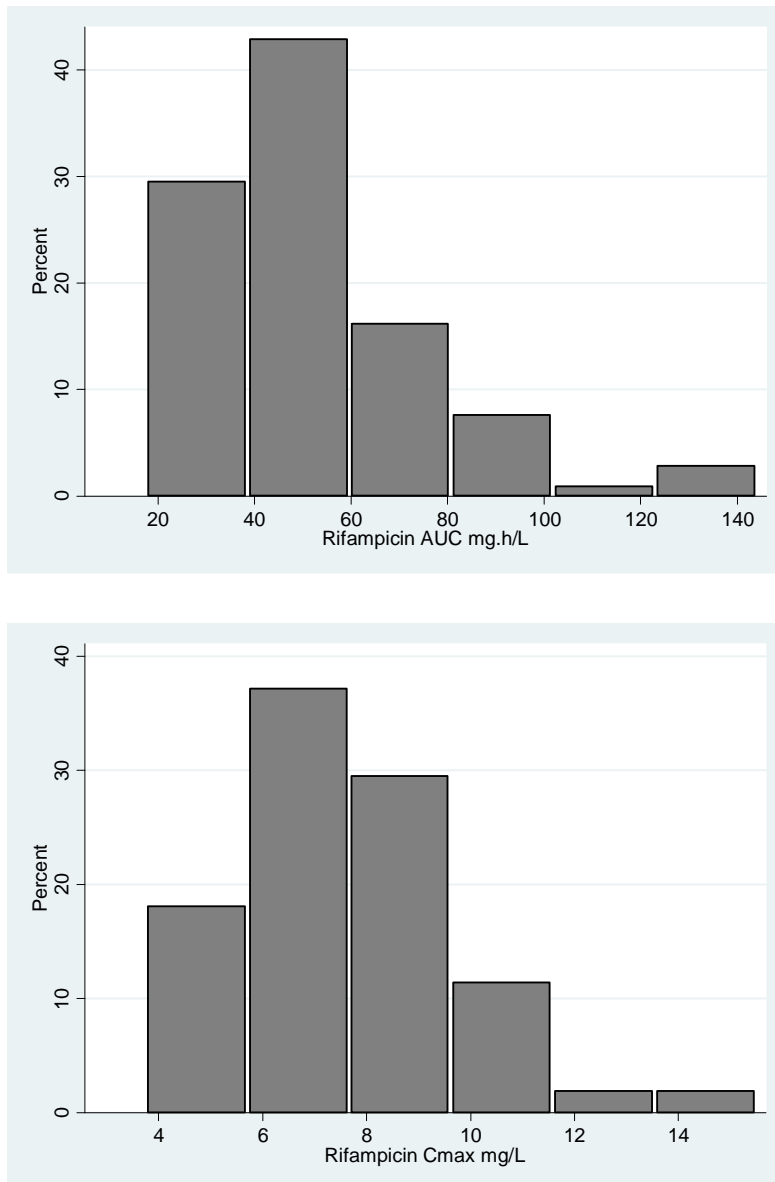


Figure 5.7: Histograms of rifampicin AUC<sub>0-24</sub> (top) and C<sub>max</sub> (bottom) in South African patients with TB

Out of the 75 patients in the dataset, 69 had MGIT culture results for the eighth week of treatment, which is the end of the intensive phase of treatment for TB. Of these 69, 34 (49%) still had the undesirable outcome of a positive sputum culture result. Stratification by sputum culture result and the number of patients with a C<sub>max</sub> above or below 8 mg/L gave the 2 x 2 contingency table below.

Table 5.4: Numbers of patients stratified by treatment outcome and rifampicin C<sub>max</sub>

C <sub>max</sub> >8 mg/L	Positive 8 week culture	Negative 8 week culture
Yes	8	16
No	26	19

A one-sided chi-square test for the above table resulted in a p-value of 0.027, with a relative risk of 0.57. This means that patients with a C<sub>max</sub> greater than 8 mg/L had a 43% lower chance of obtaining a positive 8 week culture result compared with those having a C<sub>max</sub> lower than 8 mg/L.

## 5.4 Discussion

This study is the first to show that the *SLCO1B1* rs4149032 polymorphism is associated with low rifampicin exposure. The low rifampicin concentrations in a high proportion of patients with TB is consistent with other reports of African patients (Tappero *et al.* 2005; McIlleron *et al.* 2006; Weiner *et al.* 2010). Simulations showed that a 30% dose increase of rifampicin in patients with the polymorphism would improve the proportion of patients with therapeutic rifampicin concentrations. Lower rifampicin concentrations observed in Africans could be accounted for by the *SLCO1B1* rs4149032 polymorphism, which has been reported to occur at a frequency of 75% in the Yoruba from Nigeria (and 70% in this study population), compared with 29% in Caucasians and 56% in Asians (NCBI 2010). The high allele frequency of the *SLCO1B1* rs4149032 polymorphism amongst South Africans suggests that higher doses of rifampicin may be appropriate for South African populations. Increasing the rifampicin dose could improve sputum culture conversion rates, which have been reported to be lower in Africans compared with non-Africans in two multicentre studies (Burman *et al.* 2006; Dorman *et al.* 2009). There is *in vitro*, animal and human evidence that increasing the rifampicin dose improves its antituberculosis activity, and higher doses are unlikely to result

in a significant increase in adverse events (Mitnick *et al.* 2009). Currently, genotyping prior to treatment would be difficult to implement, particularly in the current resource limited setting. Since the majority of the South Africans in this study were carriers of the rs4149032 polymorphism, increasing the dose of rifampicin across the board will benefit not only those with the polymorphism, but also possibly those without the polymorphism as higher doses are likely to be more effective and appear to be safe.

Pertaining to the 18 patients who did not have DNA available for genotyping, the mixture model predicted a proportion of 0.07 of these patients (in fact 1 patient) not to be carriers of the rs4149032 polymorphism. This is comparable to the value of 0.12 for those who did have genotype results. The large RSE on the mixing probability in table 5.3 is probably due to the small number of patients with missing covariate data combined with the low frequency of wild type individuals thus creating difficulties in estimation. An attempt to divide the 18 patients into 3 subpopulations (wild type, heterozygotes and homozygotes) using a 3 mode mixture model rather than 2 did not improve the OFV and created model instability, hence the patients were divided only into carriers and non-carriers (wild type) of the SNP.

An investigation was carried out to determine the effect of several polymorphisms of drug transporters and transcriptional regulators in an attempt to explain the high variability in rifampicin pharmacokinetics that is evident from the histograms of the AUC and C<sub>max</sub> in the current study and has been reported previously (Wilkins *et al.* 2008). However, it must be noted that only two or three homozygotes for each of the SNPs were included in the present study. Therefore, the contribution of the SNPs needs to be assessed in a larger cohort. For *PXR*, it is possible that a pharmacogenetic effect was not observed because the induction of *PXR* by rifampicin may have overridden any potential genetic effects. Indeed, the amount of

*PXR* induction that occurs due to administration of rifampicin has been shown to be inversely proportional to the baseline *PXR* activity (Lamba *et al.* 2008). The trend observed in increased CL/F for *PXR C63396T* carriers is however consistent with the decrease found in atazanavir plasma concentrations for individuals with this polymorphism (Siccardi *et al.* 2008). High P-gp activity may limit distribution of rifampicin in the body, leading to lower V/F and higher plasma concentrations as has been previously suggested for digoxin (Sakaeda *et al.* 2001). However, women were found not to have higher CL/F than men which indicates that the role of p-glycoprotein may be more important in determining the distribution volume than the extent of oral absorption. The increase in CL/F and mean transit time combined with a decrease in V/F seen with the *ABCB1 G2677T/A* polymorphism, though not statistically significant, is suggestive of this SNP resulting in higher P-gp activity and/or expression, which is consistent with previous reports (Moriya *et al.* 2002).

An important finding was the effect of the *SLCO1B1* rs4149032 polymorphism on rifampicin exposure. This SNP significantly lowered bioavailability in the study population. This intronic SNP has recently been shown (in Caucasians) to be in LD with the functional variant *C463A* (rs11045819) termed *SLCO1B1*\*4 (Lubomirov *et al.* 2010). LD data of *SLCO1B1* gene from the HapMap project shows that there is stronger LD in Caucasians than in Africans (International Hapmap Project 2010). Different patterns of LD in Africans have been attributed to greater genetic diversity as a result of migration, admixture with other African and non African population as well as the longer lineage of the African population (Tishkoff and Williams 2002). This could explain why *C463A* may not be correlated with rs4149032 in our African population as suggested by haplotype analysis. Recently *C463A* has been shown to decrease rifampicin AUC by 36% in a mixed cohort of Africans and Caucasians (Weiner *et al.* 2010). However, this allele was uncommon in this small study population.

The shorter mean transit time in individuals receiving higher doses suggests saturation of the transporter mediated mechanisms involved in absorption (P-gp), first pass effect (P-gp and OATP1B1) and elimination (P-gp and OATP1B1) and is in agreement with the dose-dependent rifampicin pharmacokinetics reported in a previous study (Agrawal *et al.* 2004). A mixed order elimination model was evaluated during model development for this current dataset; however this failed to significantly improve the model.

Another important finding is the result that patients with a rifampicin C<sub>max</sub> greater than 8 mg/L were more likely to have a negative 8 week sputum culture result than patients with lower C<sub>max</sub> concentrations. This is one of few reports to identify differences in sputum conversion rates based on individual measures of drug exposure in a clinical setting although further analyses are required to identify a definitive quantitative concentration-effect relationship.

In conclusion, the *SLCO1B1* rs4149032 polymorphism existed at a high frequency in the South African population and was associated with low rifampicin concentrations (<8 mg/L) and therefore a lower likelihood of sputum conversion after 8 weeks of treatment. Because of the high frequency of the carrier state in Cape Town (70%) a non-selective increase in daily dose of 150 mg would nearly double the number of patients (38% to 68%) attaining the target concentration of 8 mg/L. The public health implication of this study is that an increased dose of rifampicin should be considered in South Africans. Even then 32% of patients can be expected to have concentrations below the target, meaning that even higher doses (than the increment of 150 mg) should be considered in order to enable at least 90% of patients to reach the target. However, the toxicity of higher rifampicin doses should first be considered.



## **6. PARALLEL FIRST ORDER AND MIXED ORDER ELIMINATION OF PYRAZINAMIDE IN SOUTH AFRICAN PATIENTS WITH TUBERCULOSIS**

### **6.1 Introduction**

Pyrazinamide is an important antitubercular drug with good activity against slow-growing bacilli, thus making it a key sterilizing drug (Zhang *et al.* 2003). The current recommended dose of pyrazinamide is about 25-30 mg/kg once daily (WHO 2008). The AUC:MIC ratio has been shown to be an important predictor of the sterilizing effect of pyrazinamide and doses at a minimum of 60 mg/kg have been shown to perform better than lower doses, based on linear pharmacokinetics (Gumbo *et al.* 2009). It is not surprising therefore that clinical investigation of higher doses has been suggested in recent times (Pasipanodya and Gumbo 2010). These suggestions and extrapolations have been made based on first order elimination of pyrazinamide, which is what is generally reported in the literature.

Pyrazinamide has been found to be eliminated renally (about 3%), and the rest as various metabolites through multiple metabolic pathways (Lacroix *et al.* 1989). A zero-order input model with first order elimination of pyrazinamide administered to patients in the fasted state (Wilkins *et al.* 2006) poorly described the data, particularly in the absorption phase of the current cohort of South African patients with TB. This may be due to model misspecification for pyrazinamide absorption in that model, or the different fixed dose combination formulation being used in this new cohort resulting different absorption properties, or concomitant administration of food in this study altering the absorption properties. The current objective was therefore to adequately describe pyrazinamide pharmacokinetics in this group of patients and obtain individual measures of drug exposure (AUC and C<sub>max</sub>) for use in subsequent analyses.

## 6.2 Methods

The group of study participants were being treated for ordinary TB as outlined in the methods section 4.1. The drug concentrations were log-transformed to address numerical difficulties in the integration routine that were encountered during the model development which were resulting in premature termination of the estimation process due to exceeding the maximum number of evaluations of the differential equations. Various absorption models were investigated including first order absorption, zero order absorption, and sequential zero order and first order absorption. Elimination models tested included first order elimination, mixed order (saturable) elimination and a combination of the two in parallel. Allometric scaling was used to incorporate bodyweight on model parameters related to clearance and volume (Anderson and Holford 2008). A nonparametric bootstrap implemented using PsN was performed to obtain confidence intervals of the parameter estimates from the final model. Two-hundred replicates were performed. A separate dataset from another independent study was available for use as an external evaluation procedure of the final model through a VPC. After an estimation process, a nonparametric bootstrap of 200 replicates was done for this model as well.

Deterministic simulations were then carried out using the final model to compare drug concentration-time profiles for a dose of 30 mg/kg (dose was about 25 mg/kg in the current study) with those for the higher suggested doses (doubled to 60 mg/kg). The steady state doses used were 1500 mg and 3000 mg, both in a male individual weighing 50 kg.

## 6.3 Results

Seventy-six patients gave a total of 614 observations. Two patients were excluded because they did not have pyrazinamide concentration-time data available. Of the 614 observations, 2 were below the LLOQ and were excluded from the analysis.

Table 6.1 outlines the steps taken through the model building for pyrazinamide.

Table 6.1: Summary table of pyrazinamide population model development

Model	Description	OFV	Comment
1	One compartment, combined zero order and first order absorption, first order elimination. Adapted from Wilkins <i>et al</i> , 2006. PPV on Cl, V, KA, F	-159	Appears to be some misspecification (Figure 6.1a), perhaps due to administration of food in the current study compared to a fasted state for Wilkins <i>et al</i> , or different formulations.
2	Test lag time with first order absorption instead of zero order absorption from model 1	-170	
3	Test sequential dual absorption rate constant instead of lag time from model 2. Slow absorption early on followed by more rapid absorption later	-178	More physiological than lag time which is just right shift of the concentration-time profile.
4	Test time dependent proportional error model from model 3 for to cater for potentially higher uncertainty in early time points followed by lower error when the rate of change of concentrations with respect to time is lower (Karlsson <i>et al</i> . 1995). Change point of 0.5 h from high error to low error	-248	82% error for first 0.5 h, followed by 35% error thereafter. Both very high.
5	Change point of 1 h from model 4	-258	72% error for first 1 h, followed by 34% error thereafter.
6	Change point of 1.5 h from model 4	-285	80% error for first 1.5 h, followed by 31% error thereafter. Both errors still high.
7	Test mixed order elimination instead of first order elimination from model 6	-303	V <sub>max</sub> estimate of $1.7 \times 10^6$ mg/h and K <sub>m</sub> of 491 000 mg/L both very high, suggesting a component of first order elimination. But early error decreased to 72%.
8	Add first order clearance to model 7	-310	Reasonable V <sub>max</sub> estimate of 7.8 mg/h and K <sub>m</sub> of 0.8 mg/L.

9	Add allometric scaling to both clearances and volume from model 8	-320	Variability in V went towards zero and was subsequently removed. Remain with variability on Cl, F and KA.
10	Add variability in time of change from low KA to high KA for the sequential absorption rates from model 9	-331	
11	Add within subject variability in Ka to model 10	-424	
12	Add within subject variability to Cl from model 11	-430	
13	Add within subject variability to time of change from low to high KA	-678	Significant improvement.
14	From model 13, test sex on V as found by Wilkins <i>et al</i> (2006)	-704	Males have higher volume than females.
15	From model 13, test sex on Cl	-684	Males have higher clearance than females.
16	From model 13, test sex on F	-722	Males have lower bioavailability than females. Final model.

The model that best described the data was comprised of a sequential, dual first order process to describe drug absorption. In other words, a time-dependent first order absorption rate constant characterised the absorption of pyrazinamide, with very slow absorption ( $k_a=0.02 \text{ h}^{-1}$ ) taking place for the first 0.7 h, followed by more rapid absorption ( $k_a=1.0 \text{ h}^{-1}$ ) thereafter. A one compartment model with a combination of first order and mixed order elimination in parallel best described the disposition of pyrazinamide. A time dependent residual error model was incorporated to account for changes in the residual error with time, before 1.5 h after the dose (higher error) and after 1.5 h (lower error). This error was additive in the log domain which approximates to proportional in the untransformed domain. Bioavailability was found to be higher in females than in males. BSV and WSV were included on the total elimination of pyrazinamide, as well as on the time point at which the absorption rate increased. There was modest variability in bioavailability (16%), which had the typical value fixed to 1, whilst significant WSV in the higher absorption rate constant was observed. The final control stream can be seen in appendix 3.2. Table 6.2 summarizes the final model parameter estimates and their confidence intervals.

Table 6.2: Pyrazinamide population pharmacokinetic model parameter estimates

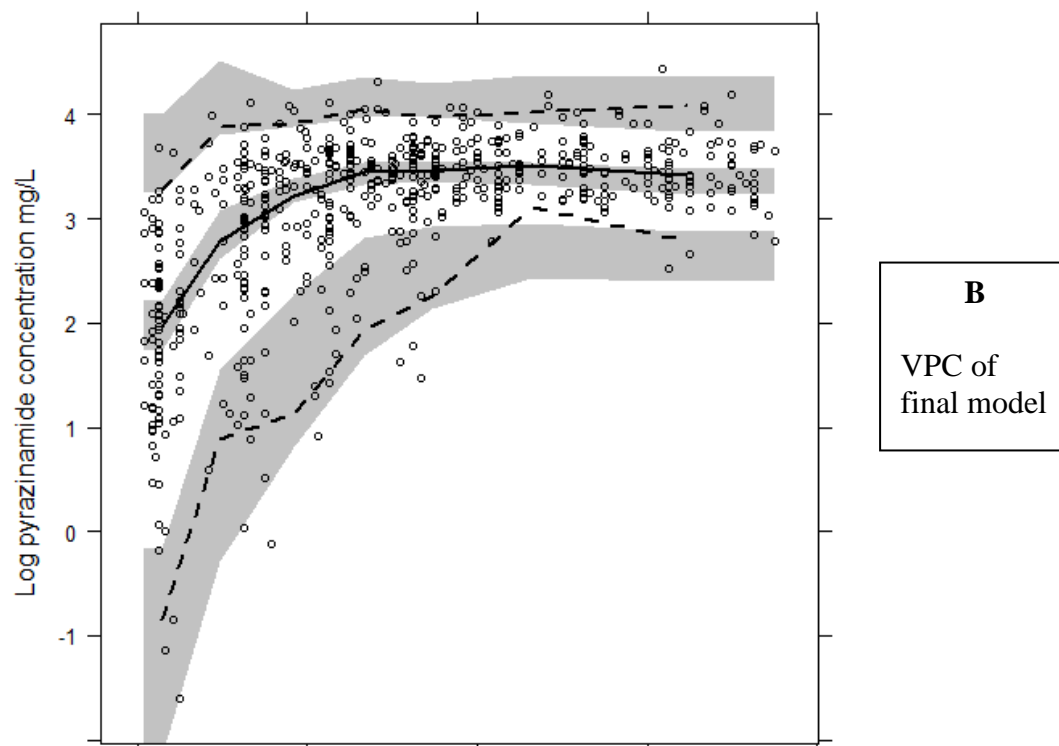
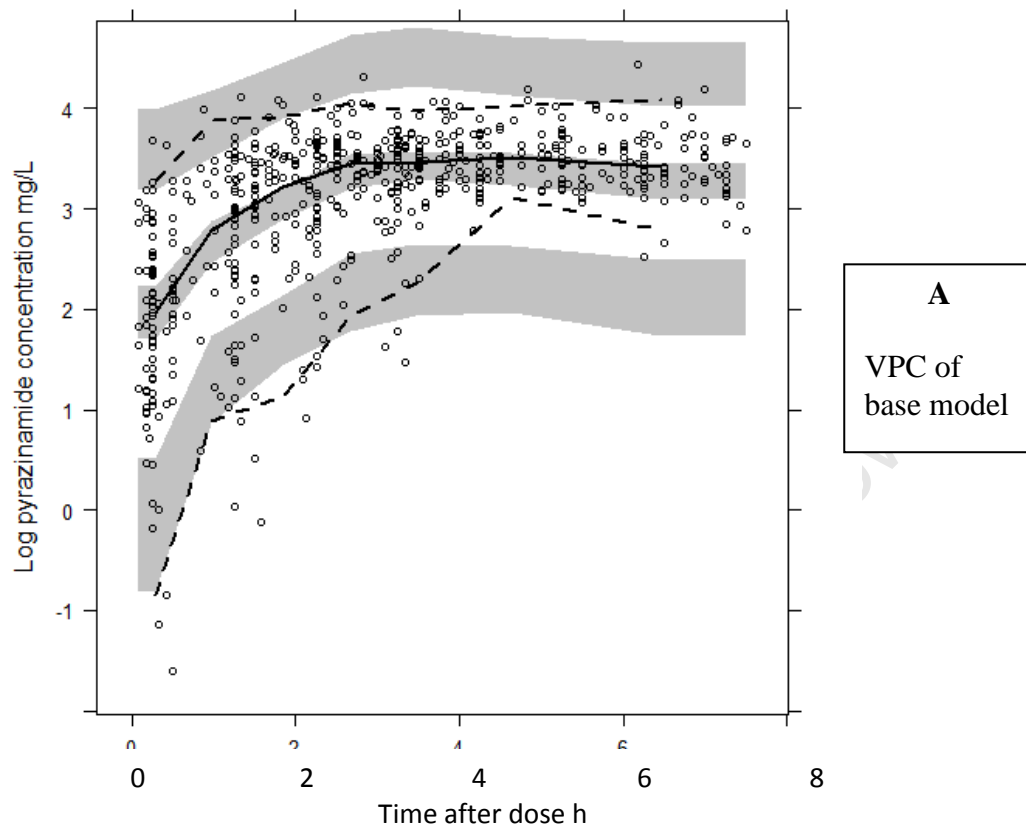
Parameter	Estimate	95% Confidence Interval
First order oral clearance L/h/70kg	2.6	2.3-3.0
$V_{\max}$ mg/h/70kg*	14.3	11.2-15.8
$K_m$ mg/L*	0.5	0.3-1.9
Early $K_a$ h <sup>-1</sup> *	0.02	0.01-0.03
Late $K_a$ h <sup>-1</sup>	1.0	0.7-1.1
Change point for $K_a$ h	0.7	0.67-0.97
Volume L/70kg	42	37-44
Effect of female sex on oral bioavailability %	+26	19-33
Proportional error (up to 1.5 h after the dose) %	42	31-44
Proportional error (from 1.5 h after the dose) %	14	10-16
BSV* for combined elimination %	17	16-23
WSV* for combined elimination %	16	14-19
BSV for change point in $K_a$ %	45	43-55
WSV for change point in $K_a$ %	48	45-69
WSV for late $K_a$ %	82	70-94
BSV for bioavailability	16%	13-19

\* $V_{\max}$  – Maximum elimination rate for first order process,  $K_m$  – drug concentration that results in half  $V_{\max}$ ,  $K_a$  – first order absorption rate constant, BSV – between subject variability, WSV – within subject variability

The average steady state pyrazinamide plasma concentration (average of all the concentrations in the dataset) was 26 mg/L. This means that on average, 17% of pyrazinamide elimination is mixed order, with the remaining majority being first order.

A VPC of the final model showed that the model could predict the original data very well. A VPC of the model after estimation on the external dataset also shows that the same model is

suitable for application on different datasets. These VPCs, in addition to that of the base model are found below in figure 6.1a, b and c.



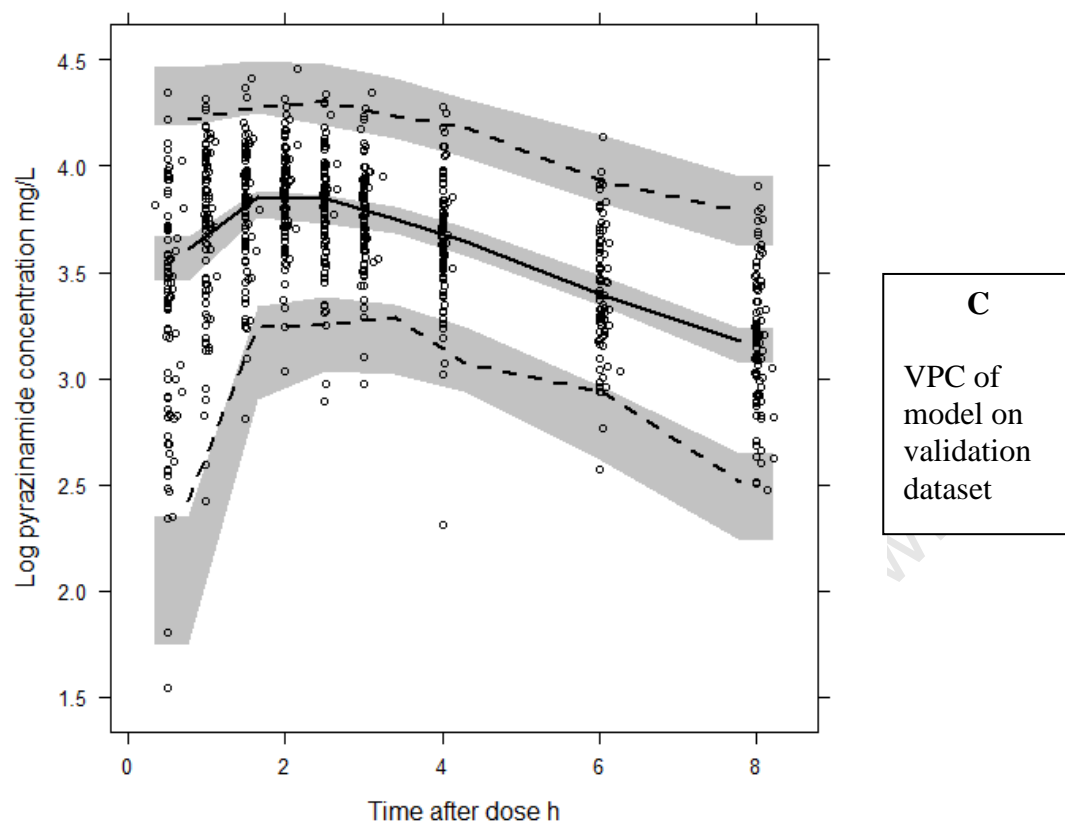


Figure 6.1: Top (A) – VPC of base model adapted from Wilkins et al, 2006. Middle (B) – VPC of final model on the model-building dataset. Bottom (C) – VPC of final model after estimation on external evaluation dataset.

The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

The parameter estimates of the model after being run on the validation dataset are shown below.

Table 6.3: Parameter estimates of final model on validation dataset

Parameter	Estimate	95% Confidence Interval
First order oral clearance L/h/70kg	3.9	3.0-5.6
$V_{\max}$ mg/h/70kg	18.6	0.8-38
$K_m$ mg/L	2.4	0.2-5.0
Early $K_a$ h <sup>-1</sup>	0.16	0.05-0.27
Late $K_a$ h <sup>-1</sup>	2.2	1.7-2.8
Change point for $K_a$ h	0.24	0.16-0.31
Volume L/70kg	39	34-54
Proportional error (up to 1.5 h after the dose) %	13	10-17
Proportional error (from 1.5 h after the dose) %	11	7-15
PPV* for combined elimination %	19	12-24
PPV for change point in $K_a$ %	61	32-98
PPV for late $K_a$ %	80	62-98
PPV for bioavailability %	17	13-22

\* PPV – population variability. As this dataset only had 1 occasion, PPV is thus a summation of BSV and WSV.

The parameter estimates are similar to those of the original dataset, except that absorption occurs much faster in this group of patients, and the effect of sex on bioavailability could not be identified. Females were estimated to have a bioavailability 3% higher than males but this was not significant and was removed from the model used for the bootstrap.

Returning to the patients in the current study, the final model was used to obtain the summary pharmacokinetic measures of the  $AUC_{0-24}$  and the  $C_{\max}$ , histograms of which are shown below.



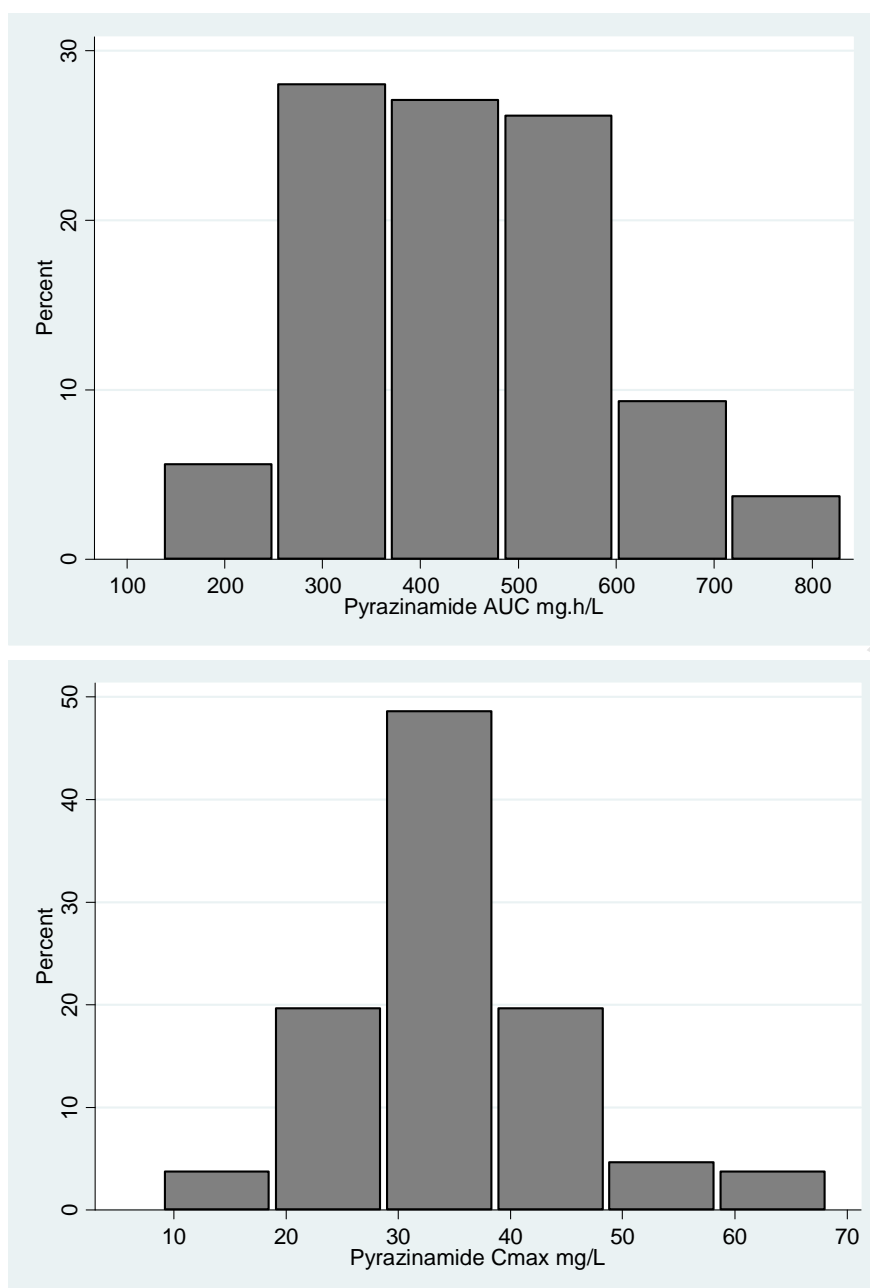


Figure 6.2: Histograms of pyrazinamide AUC<sub>0-24</sub> and C<sub>max</sub> in South African patients with TB

The median (interquartile range) of the AUC<sub>0-24</sub> and C<sub>max</sub> were 418 (339-528) mg.h/L and 34 (29-39) mg/L respectively.

Figure 6.3 below shows the results of the deterministic simulations performed using the final model at 2 weight-based dose levels. In order to compare the consequences of assuming first

order elimination, the final model was once again reduced to a model with first order elimination. This was accompanied by a 30 point increase in the OFV and the first order clearance was estimated to be 4.1 L/h, although other model parameters remained similar to those of the full model (except for the early proportional error which increased to 58%).

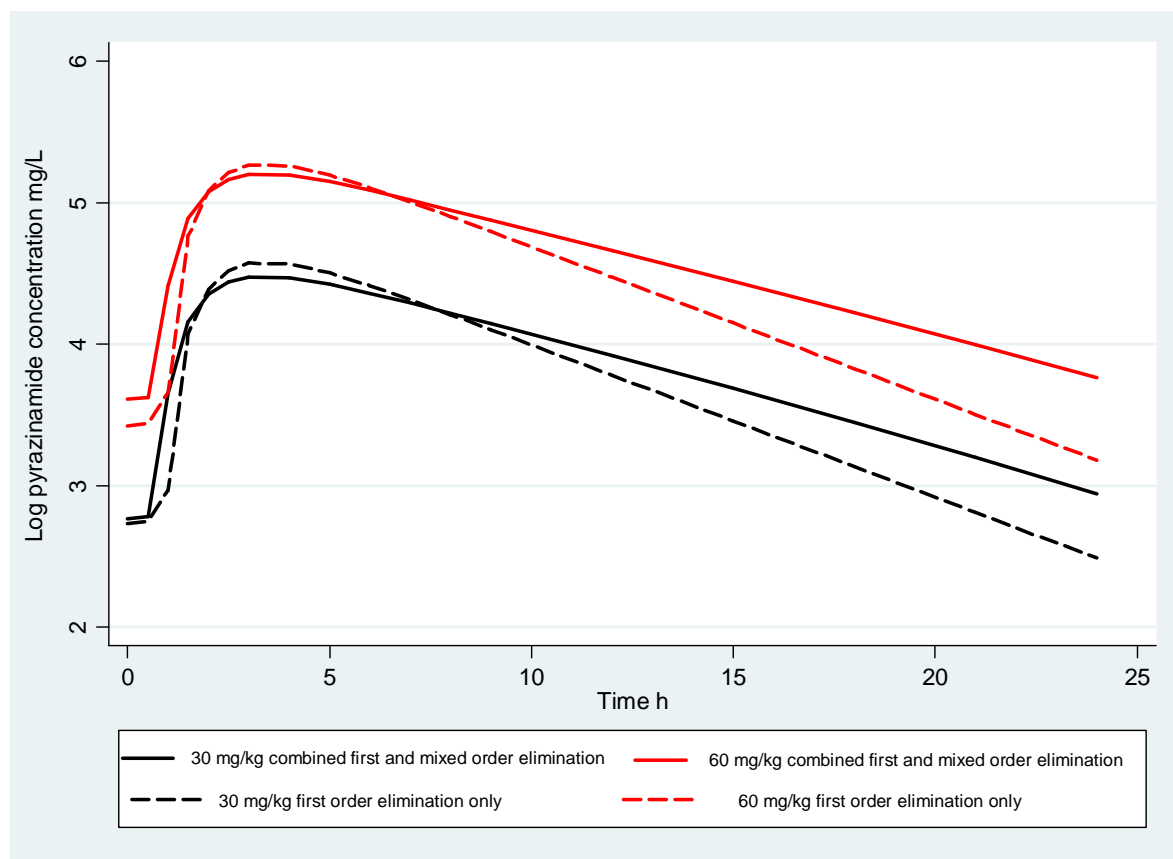


Figure 6.3: Comparison of pyrazinamide plasma concentration-time profiles from the final model (combined first order and mixed order elimination) and a model assuming first order elimination only.

Figure 6.3 illustrates significant differences in the concentration-time profiles for the different models, with the differences becoming more pronounced for the higher dose (red lines). For the 1500 mg dose (30 mg/kg), the predicted  $AUC_{0-24}$  was 577 mg.h/L based on the model with parallel first order and mixed order elimination, compared to 511 mg.h/L when

assuming first order elimination only. These values were higher than the median observed AUC because they were simulated using a 30 mg/kg dose (for ease of comparison with a 60 mg/kg dose), rather than 25 mg/kg dose. For the 3000 mg dose, the  $AUC_{0-24}$  increased to 1277 mg.h/L based on the model with parallel first order and mixed order elimination, compared to 1023 mg.h/L for the model assuming first order elimination only. This means that assuming first order elimination only will result in underestimation of the overall exposure by 25% for the 3000 mg (60 mg/kg) dose.

## 6.4 Discussion

The pharmacokinetics of pyrazinamide was adequately described taking into account parallel first order and mixed order (saturable) elimination, together with time-dependent first order absorption. Since pyrazinamide is eliminated through various pathways in the body (Lacroix *et al.* 1989), the current model suggests that one or more of these pathways may be saturable. The saturable pathway was responsible for elimination of about one-fifth of the average observed steady state concentrations in this study; although this proportion will increase if higher doses are given. The reduced model had a first order clearance of 4.1 L/h which is similar to 3.4 L/h reported in previous models (Wilkins *et al.* 2006).

The precision of the parameter estimates and the ability of the model to be applied to a new dataset is testament of the robustness of the model. However, the confidence interval of the  $K_m$  was wide therefore the estimate of the  $K_m$  should be interpreted with caution. Having pharmacokinetic observations well beyond 7 hours after the dose would enable better precision in the estimation of  $K_m$ . High variability was observed in the absorption parameters, although variability in bioavailability and clearance was low. This high absorption variability is probably without consequence as the total daily exposure remains unchanged and

pyrazinamide efficacy is driven by the AUC (Gumbo *et al.* 2009). Pyrazinamide generally appears to be a less variable drug than rifampicin and isoniazid. The near-zero absorption rate for the first 0.7 h after the dose has been administered was most probably an absorption delay before the drug becomes available for absorption at the absorption site in the small intestine.

Males were found to have a lower bioavailability of pyrazinamide compared to females. Another study found that males had a 15% greater volume of distribution compared to females (Wilkins *et al.* 2006). During the model development process, males were found not only to have a greater volume (V/F), but also a greater clearance (CL/F) after weight had already been added to the covariate model, which led to an investigation of the effect of sex on bioavailability (F). The model with sex on bioavailability was subsequently chosen because it had an OFV 24 points lower than the former, with one less parameter.

With increasing calls for dose-optimization of antitubercular drugs (Gumbo 2010), accurate descriptions of the pharmacokinetics of the drugs are required to justify extrapolations of doses before clinical studies are undertaken. This model shows that doubling the current recommended dose of pyrazinamide can result in significant underestimation of the predicted AUC and possibly lead to administration of toxic doses. The magnitude of this underestimation can only increase if predictions for even higher doses are made assuming linear elimination.

## 6.5 Conclusion

To our knowledge, this is the first report describing mixed order elimination of pyrazinamide, let alone first order and mixed order elimination in parallel. This means that pyrazinamide exhibits dose-dependent pharmacokinetics which must be taken into account when

investigating doses higher than those currently in use. The model enabled accurate determination of individual patient measures of drug exposure (AUC and C<sub>max</sub>) to be used in later analyses.

University of Cape Town

## 7. PHARMACOKINETICS OF ISONIAZID IN SOUTH AFRICAN PATIENTS WITH TUBERCULOSIS

### 7.1 Introduction

Isoniazid is an antitubercular drug with high EBA and is responsible for killing a significant proportion of actively dividing bacilli in the first few days of treatment in patients. The literature contains numerous studies of the influence of NAT-2 polymorphism on the clearance of isoniazid. This is one of the earliest and most studied gene-drug interactions and is one of the classical examples in the field of pharmacogenetics. Thus investigation of the effect of NAT-2 polymorphism was outside the scope of this work which sought to obtain individual measures of isoniazid exposure (AUC and C<sub>max</sub>) through a NLME approach for use in subsequent analyses.

### 7.2 Methods

The group of study participants were being treated for ordinary TB as outlined in the methods section 4.1. The final model was modified from a published model in a similar group of patients (Wilkins *et al.* 2011). Wilkins *et al* described isoniazid pharmacokinetics through a 2 compartment model with first order absorption and first order elimination. They also used a mixture model to distinguish between the clearance of slow and fast acetylators. In this work, Wilkins *et al*'s model was modified based on sound biological principles to achieve a reasonable description of the observed data. Isoniazid concentrations were log-transformed after experiencing numerical difficulties during the model building. Beal's M3 method (Ahn *et al.* 2008; Beal, 2001) was used to handle data below the LLOQ. The VPC was stratified to predict uncensored observations (above the LLOQ) as usual, in addition to making a

comparison of the proportion of predictions which were below the LLOQ with the proportion of the observations actually below the LLOQ for each bin.

### 7.3 Results

Seventy-eight patients provided a total of 634 observations. Eighty-four (13%) of these observations were below the LLOQ of 0.1 mg/L, and were mostly in the absorption phase. Table 7.1 below outlines the model building process from Wilkins *et al*'s model until the final model was reached.

Table 7.1: Summary table of isoniazid population model development

Model	Description	OFV	Comment
1	Modified from Wilkins <i>et al</i> (2011). Implemented a 2-compartment model with first order elimination and absorption. Mixture model on clearance. F fixed to 1. Allometric scaling for body weight and all clearances and volumes. BSV on CL, V, KA and F. WSV on F and KA	331	Intercompartmental clearance (Q) estimate of $2.27 \times 10^8$ . Short sampling schedule of only up to 7 hours may compromise ability to identify 2 compartments. Model was unable to identify 2 subpopulations with mixing probability going to the boundary of 1
2	From model 1, fix Q and peripheral volume to 3.34 L/h and 1730 L respectively (values from Wilkins <i>et al</i> )	327	Mixing probability now estimated to be 0.76. However, high proportional error of 62% needs to be addressed
3	From model 2, try one compartment model instead of 2 compartments.	323	The data does not seem to support a 2-compartment model
4	From model 3, test transit compartment absorption model	421	Likely a local minimum, and OFV was sensitive to initial estimates. Removed BSV and WSV in F to reduce number of parameters
5	From model 4, replace WSV in KA with WSV in MTT.	71	Significant improvement. Model is more stable with variability on MTT
5	Add WSV in CL to model 4	-28	
6	Add WSV in V to model 5	-41	
7	Add covariance between BSV CL and BSV V to model 6	-96	Final model
8	Add covariance between WSV CL and WSV V to model 7	-96	Not significant. Model does not run very well. Changing initial

			estimates results in higher OFV, hence it is prone to local minima.
9	To model 7, add bioavailability to mixture model i.e. individual with low CL will have F fixed to 1, whilst the F for individuals with high CL is estimated	-98	Not significant

Implementation of a 2-compartment model and estimating the parameters resulted in an unphysiological and biologically implausible intercompartmental clearance. At this stage, the mixture model failed to identify 2 different subpopulations with respect to clearance, with the mixing probability going towards zero for one subgroup. Fixing the intercompartmental clearance and the peripheral volume of distribution to those reported by Wilkins *et al* resulted in a more realistic model and identification of bimodality in isoniazid clearance. However the high proportional error of 62% meant that further optimization of the model was required as outlined in table 7.1.

The final model was comprised of a transit compartment absorption model with a bimodal distribution for clearance, followed by a one compartment model with first order elimination. Allometric scaling was used to incorporate bodyweight on clearance and volume. A covariance between the random effects (BSV) of clearance and volume was included, which addresses variability in bioavailability. A covariance of the WSV of clearance and volume was tested but was not found to improve the model or be statistically significant. WSV in the mean transit time was also part of the final model, although the BSV in that parameter went towards zero and was subsequently removed. An additive error model (in the log-transformed domain, which approximates to a proportional error in the normal domain) was used to characterise the residual unexplained variability. The final model parameter estimates and RSE from the NONMEM covariance step are shown in table 7.2. The final model control stream is shown in appendix 3.3.



Table 7.2: Isoniazid population pharmacokinetic model parameter estimates

Parameter	Estimate (%RSE)
Clearance in fast eliminators L/h/70 kg	25 (21)
Clearance in slow eliminators L/h/70 kg	13 (25)
Proportion of fast eliminators in population	0.54 (46)
Volume L/70 kg	126 (13)
Number of transit compartments	10 (Fixed)
Mean transit time h	0.7 (11)
First order absorption rate constant $k_a \text{ h}^{-1}$	3.6 (0.01)
Relative bioavailability	1 (Fixed)
Proportional error %	24 (2.6)
BSV in clearance %	53 (11)
BSV in volume %	76 (17)
Correlation between BSV clearance and volume	0.78 (13)
WSV in clearance %	30 (14)
WSV in volume %	35 (36)
WSV in mean transit time	120 (9)

A VPC below of the final model shows that the model can accurately predict the data in terms of central tendency and variability. In addition the final model can correctly predict the proportion of data that was below the LLOQ.

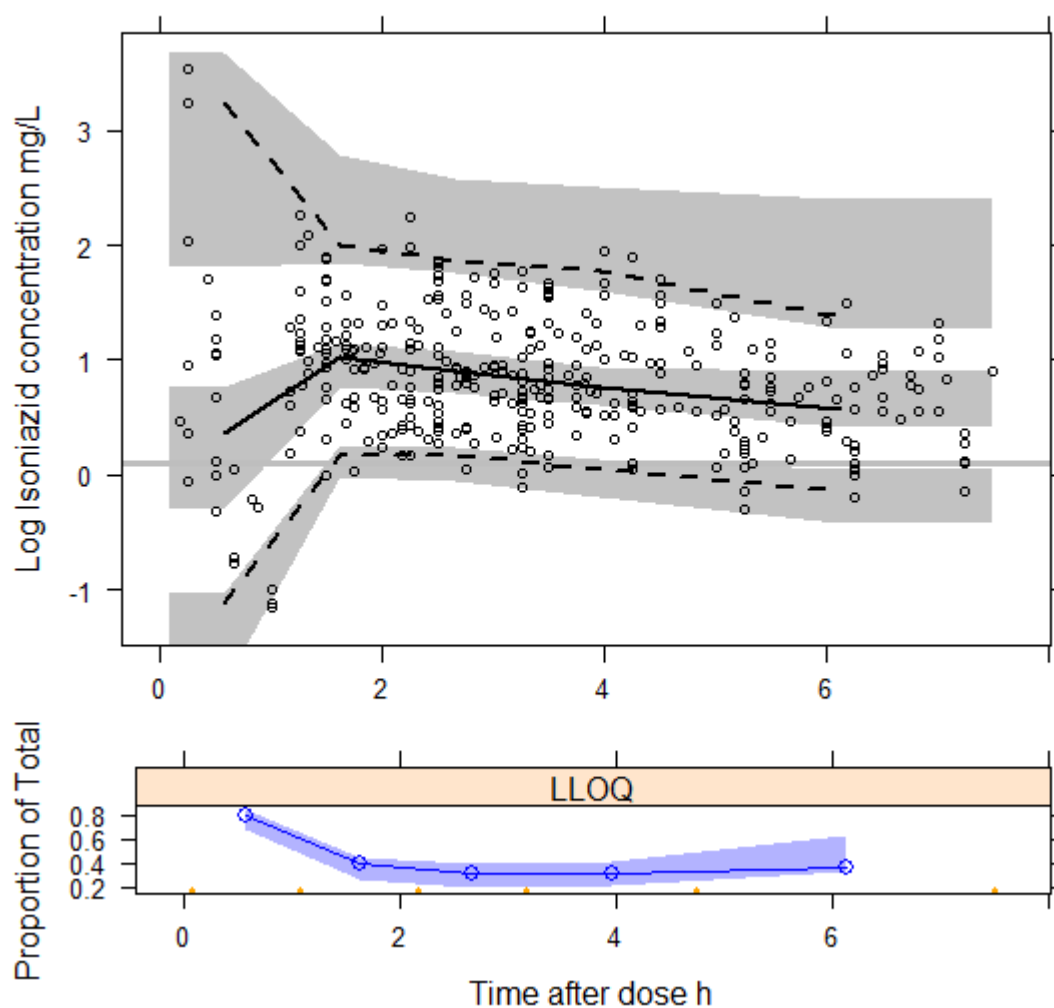


Figure 7.1: VPC of the final model for the uncensored data (upper panel) and for the data below the LLOQ (lower panel).

The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles. The blue shaded area is the 95% confidence interval of the simulated proportion of data below a concentration of 0.1 mg/L (LLOQ in untransformed domain), whilst the open blue circles represent the proportion of the observations below LLOQ.

Histograms of the  $AUC_{0-24}$  as well as for the  $C_{max}$  are shown below.

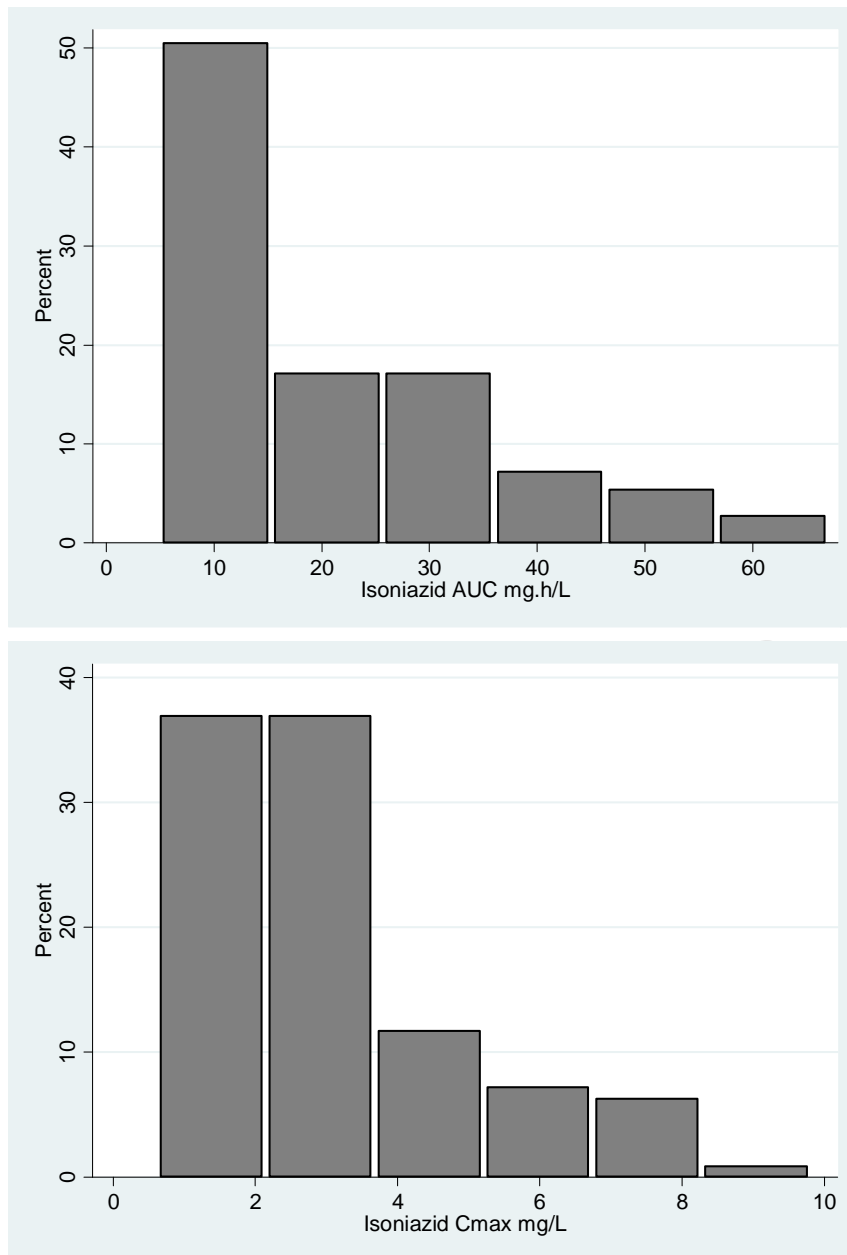


Figure 7.2: Histograms of isoniazid  $AUC_{0-24}$  and  $C_{max}$  in South African patients with TB

The median (interquartile range) for the  $AUC_{0-24}$  and  $C_{max}$  were 15 (10-28) mg.h/L and 2.6 (1.6-3.7) mg/L, respectively.

## 7.4 Discussion

The pharmacokinetics of isoniazid has been adequately described in this cohort, enabling reliable determination of the AUC and C<sub>max</sub>. Bimodality in the clearance of isoniazid was observed which is consistent with previous studies (Peloquin *et al.* 1997; Wilkins *et al.* 2011). Trimodality in isoniazid pharmacokinetics could not be supported by the data, and it is likely that the mixture model grouped probable slow acetylators together with probable intermediate acetylators together. Genetic analysis of various sub-Saharan African populations has also shown a high prevalence of slow and intermediate acetylators (Matimba *et al.* 2009), consistent with the phenotypic-based mixture model proportions. Failure to observe distribution of isoniazid into a peripheral compartment using a 2-compartment model may be due to the limited sampling schedule.

With more than 80% of the data in the first hour being below the LLOQ, it was necessary to be able to model this kind of data, which carries information though it is censored. The lower panel VPC (see figure 7.1) shows that the model could accurately predict the proportion of data that was below the LLOQ, thus implementation of Beal's M3 method was considered appropriate for this dataset.

The short mean transit time and high absorption rate constant were in agreement with previous studies that showed isoniazid absorption to be rapid (Peloquin *et al.* 1997). A high WSV in the mean transit time was observed, probably due to changes in gastric movements between occasions. Clearance was also variable probably due to NAT-2 polymorphism in the population. The estimates of the C<sub>max</sub> and AUC were consistent with what has been previously reported with isoniazid e.g. Peloquin *et al.* found C<sub>max</sub> and AUC values of 2.7 mg/L and 15 mg.h/L for individuals in the fed state (Peloquin *et al.* 1999).

## 7.5 Conclusion

The pharmacokinetics of isoniazid have been described satisfactorily and the model enabled reliable determination of the individual patient AUCs for later use in subsequent models. The bimodal distribution in clearance was likely due to NAT-2 polymorphism affecting hepatic metabolism.

University of Cape Town

## 8. PHARMACOKINETICS OF ETHAMBUTOL IN SOUTH AFRICAN PATIENTS WITH TUBERCULOSIS

### 8.1 Introduction

Ethambutol is the least potent drug in the first-line drug regimen and the objective of the modeling work presented here was to characterise its pharmacokinetics and thereafter obtain measures of drug exposure (AUC and C<sub>max</sub>) in the current cohort of South African patients with TB.

### 8.2 Methods

The group of study participants were being treated for ordinary TB as outlined in the methods section 4.1. The base model investigated was adapted from an ethambutol model in the literature built from data arising from a similar group of patients in Cape Town most of whom received the same fixed dose combination tablets (Rifafour<sup>®</sup>) (Jonsson *et al.* 2011). The literature model was comprised of a transit compartment absorption model with one transit compartment before first order absorption. The disposition was described using a 2-compartment model and first order elimination. This model was then modified based on biologically sound principles until a reasonable model for our data was obtained.

### 8.3 Results

Seventy-eight patients provided a total of 634 observations. Nine of these observations (1.4%) were below the LLOQ of 0.1 mg/L. These missing observations were imputed to half of the LLOQ value (M5 method) (Beal 2001). For the 2-compartment model, the peripheral volume of distribution was about 4000 L which is questionable and was completely different from 623 L reported by Jonsson *et al* (2011). The other parameter

estimates were also completely different and the model diagnostics showed that the fit of the literature model was not good for our dataset as can be seen from the figure 8.1.

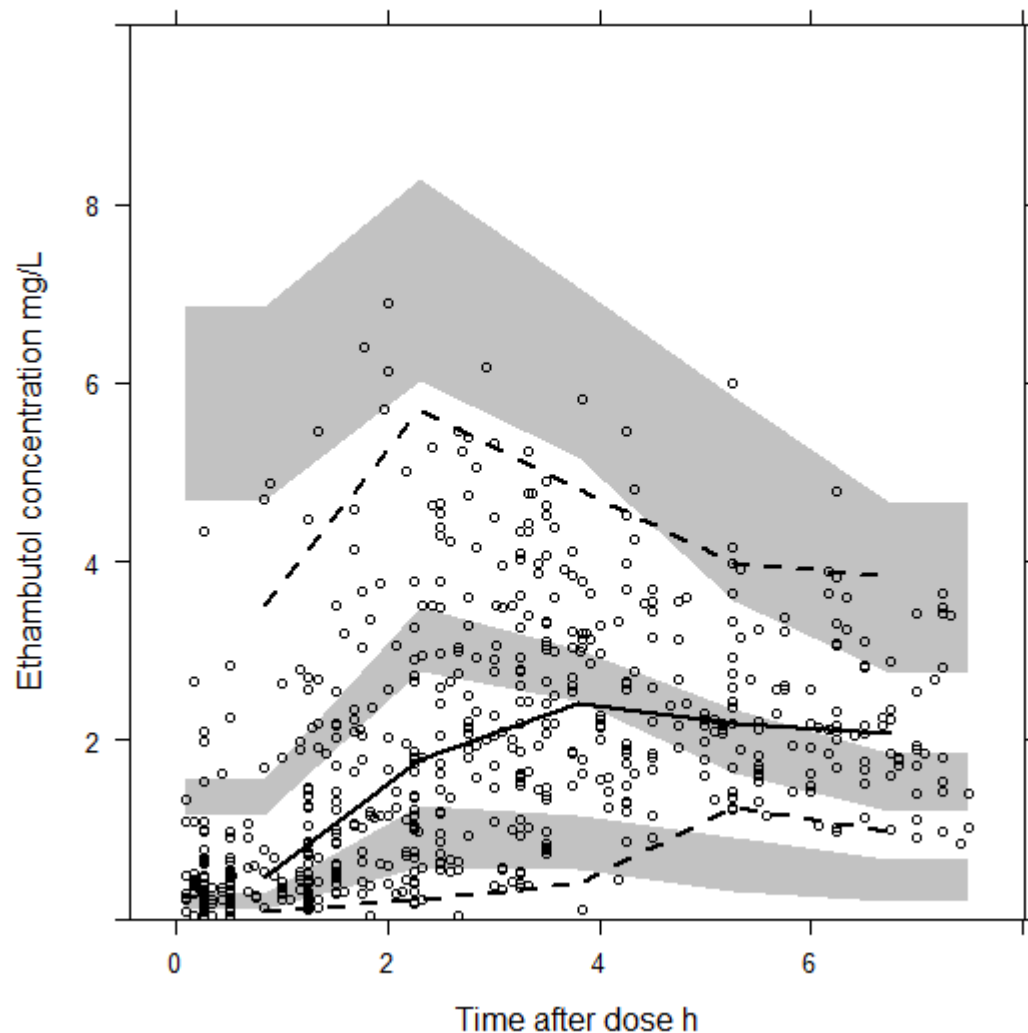


Figure 8.1: Visual predictive check of ethambutol based on model from Jonsson et al (2011) The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

Table 8.1 below summarizes the model building steps taken to reach the final model.

Table 8.1: Summary table of ethambutol population model development

Model	Description	OFV	Comment
1	Model adapted from Jonsson et al (2011). One transit compartment absorption model, followed by first order absorption and elimination. 2 compartment model. Allometric scaling on clearances and volumes. Variability on F, KA, MTT and CL.	240	Peripheral volume of 4000 L. Completely different from Jonsson's estimate of 623 L, and not expected of ethambutol. Limited sampling schedule of the current study may make estimation of 2 compartment model difficult
2	From model 1, fix intercompartmental clearance and peripheral volume to values from Jonsson et al (34.4 L/h and 623 L respectively)	303	Higher OFV
3	From model 1, try 1 compartment model	245	Hence the data cannot support a 2 compartment model
4	From model 3, test BSV and WSV on V instead of on BSV and WSV on F	217	Adding too many random effects caused local minima, hence cautious approach to limit number of random effects. Variability on V gives better fit than on F. BSV on V was not significant and was removed. Estimate of BSV for KA went to 0.3% and was also removed.
5	From model 4, instead of the 1 fixed transit compartment, try estimating the number of compartments	190	Estimate of 2.7 compartments. But WSV on KA is now 164% up from 85%
6	From model 5, test WSV on MTT instead of KA	89	Final model presented. Good VPC (figure 11.2)
7	Test HIV as covariate on F	88	Not significant. Near zero magnitude of effect.

As can be deduced from the table, subsequent modifications and model building revealed that an absorption model with an estimated 5 transit compartments, followed by first order elimination from a 1-compartment model adequately described the data. Clearance and volume were allometrically scaled for bodyweight. WSV in the absorption mean transit time, clearance and volume was included in the final model. BSV in clearance was also incorporated although BSV in other model parameters could not be supported by the data. A combined additive and proportional error model was used to describe the residual



unexplained variability. The final model parameter estimates and relative standard errors (RSE) from the NONMEM covariance step are shown in the following table. The final model control stream is shown in appendix 3.4.

Table 8.2: Ethambutol population pharmacokinetic model parameter estimates

Parameter	Estimate (%RSE)
Clearance L/h/70 kg	40 (5.5)
Volume L/70 kg	390 (6.5)
Number of transit compartments	5 (8.5)
Mean transit time h	2.2 (13)
First order absorption rate constant $k_a$ h <sup>-1</sup>	2.0 (33)
Additive error mg/L	0.06 (23)
Proportional error %	25 (4.5)
BSV in clearance %	24 (30)
WSV in clearance %	36 (13)
WSV in volume %	44 (14)
WSV in mean transit time	68 (11)

The VPC from the above final model (see figure 8.2) shows that the model can predict the data relatively well in terms of central tendency and variability.

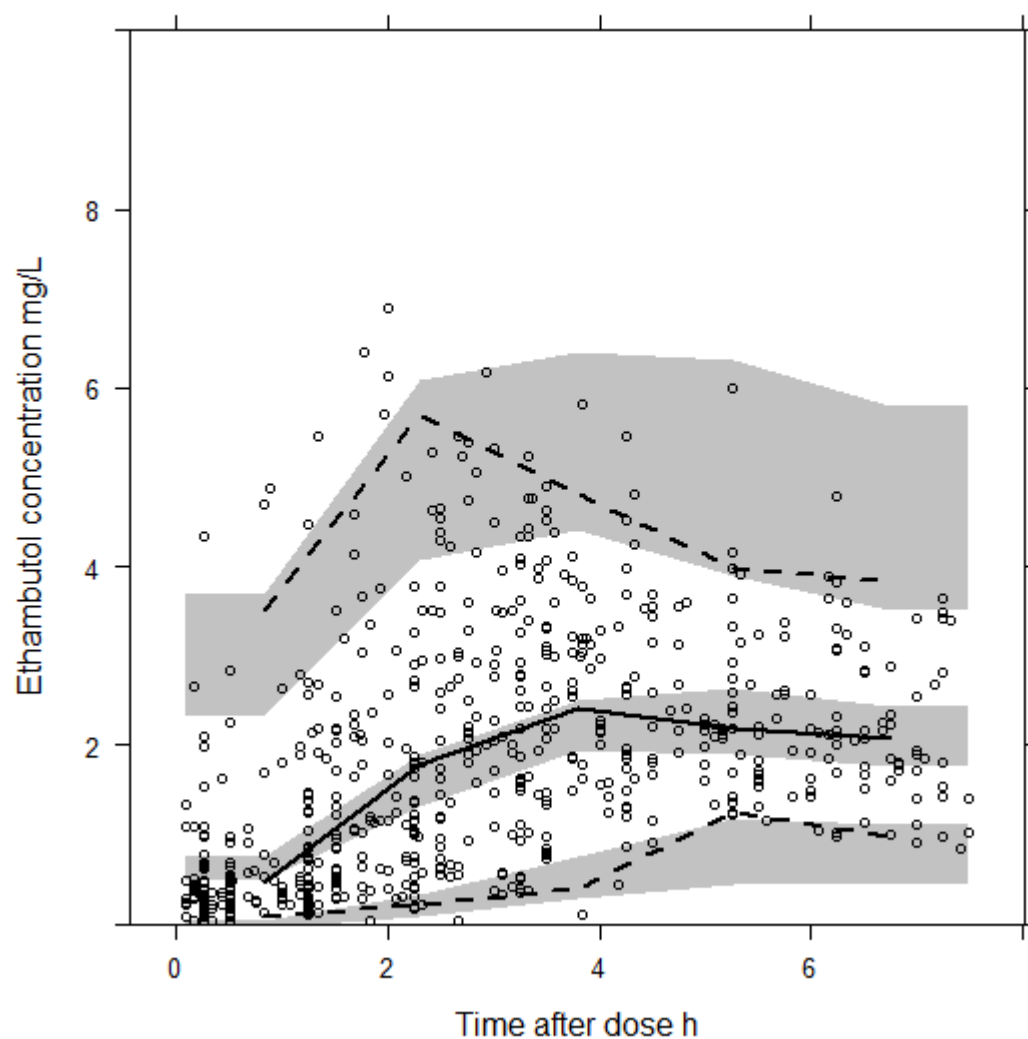


Figure 8.2: Visual predictive check of ethambutol from final model

The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

Histograms of the  $AUC_{0-24}$  as well as for the  $C_{max}$  are shown below.

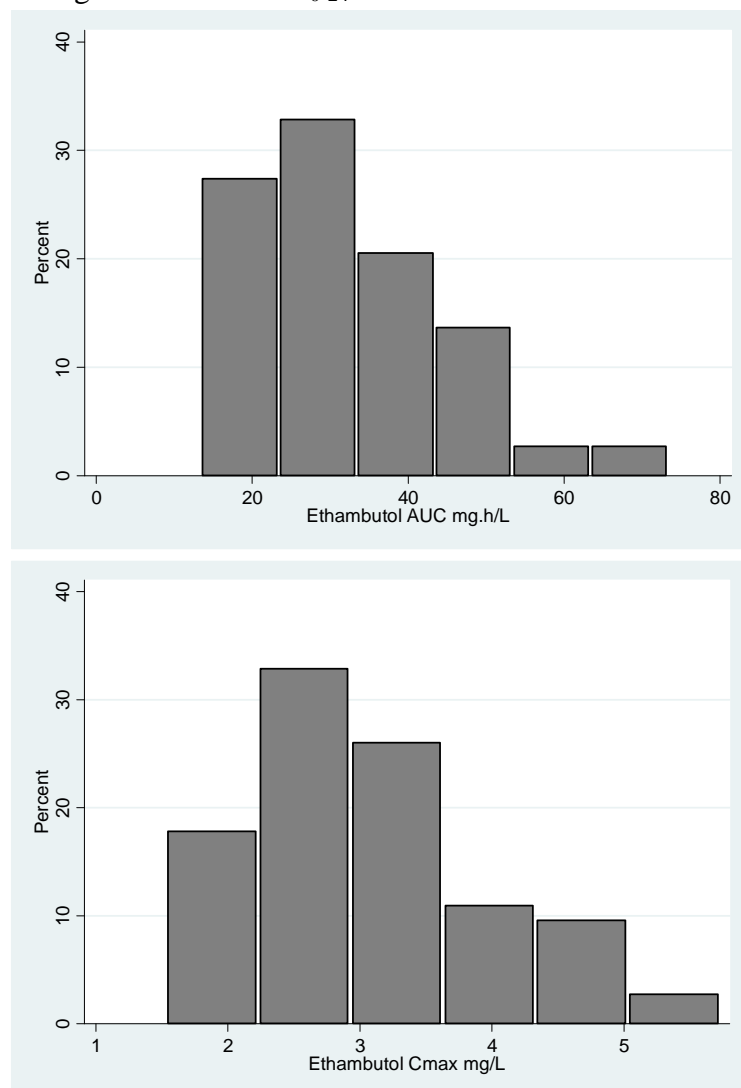


Figure 8.3: Histograms of ethambutol  $AUC_{0-24}$  and  $C_{max}$  in South African patients with TB

The median (interquartile range) for the  $AUC_{0-24}$  and  $C_{max}$  were 30 (22-38) mg.h/L and 2.9 (2.4-3.6) mg/L, respectively.

## 8.4 Discussion

In contrast to the model from Jonsson *et al* (2011) distribution of ethambutol into 2 compartments could not be demonstrated and a 1-compartment model adequately described the data. This may be due to the sampling scheme whereby the last time point was about 7

hours in the current dataset, yet Jonsson *et al* had samples up to 12 hours, coupled with faster drug absorption since Jonsson *et al*'s patients took the drug after an overnight fast. A 1-compartment model similar to the one being reported here has also been described in the literature (Zhu *et al.* 2004). The typical value of volume of distribution is very similar to that reported by Zhu *et al* who reported a value of 6 L/kg (420 L/70kg). The high volume of distribution can be interpreted to show that ethambutol distributes well into the tissues, away from the central compartment.

The estimate of clearance was very similar to that reported by Jonsson *et al* yet the 2 values were less than half the 88 L/70kg reported by Zhu *et al* who investigated a cohort of TB patients in the USA. That study cohort had approximately equal proportions of Blacks, Hispanics and Caucasians (Zhu *et al.* 2004) hence it is unclear whether the observed difference in clearance may be due to race. About half an ethambutol dose is eliminated unchanged via the kidneys, whilst another 20% is eliminated in the faeces, with the rest being metabolised (Lee *et al.* 1980). It is then not surprising that renal failure reduces the clearance of ethambutol (Varughese *et al.* 1986). Renal or hepatic dysfunction are however not a likely explanation in the current study population as patients with such conditions were excluded from the study at the outset.

A test of HIV as a covariate on bioavailability of ethambutol found HIV not to be of significance, although Jonsson *et al* mention a 15% decrease in bioavailability in HIV positive patients, which they report to be of borderline statistical significance. Failure to observe an effect of HIV may be due to the small number of patients with the condition (17%) thus the limited sample size may have compromised any ability to detect such an effect.

The disposition kinetics of ethambutol did not appear to be as highly variable as that of the other antitubercular drugs, particularly rifampicin. However, there was high variability in the absorption mean transit time which should not affect the total exposure (Peloquin *et al.* 1999).

## 8.5 Conclusion

The pharmacokinetics of ethambutol have been described satisfactorily and the model enabled reliable determination of the individual patient AUCs for later use in subsequent models. Bodyweight was a significant predictor of ethambutol clearance and volume, in agreement with literature.

## **9. POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS OF OFLOXACIN IN SOUTH AFRICAN PATIENTS WITH MULTI-DRUG RESISTANT TUBERCULOSIS**

*Antimicrobial Agents and Chemotherapy*. 2012 Jul;56(7):3857-63

### **9.1 Introduction**

Despite the important role of fluoroquinolones and the predominant use of ofloxacin for treating MDR-TB in South Africa, there are: 1) limited data on ofloxacin pharmacokinetics in patients with MDR-TB, 2) no ofloxacin pharmacokinetic data from South African patients and 3) no direct assessment of the relationship between ofloxacin pharmacokinetics and the MIC of ofloxacin of patient isolates.

Ofloxacin is primarily renally eliminated with a combination of glomerular filtration and active secretion (Lode *et al.* 1987). The respective proportions of the drug eliminated through glomerular filtration and extra-glomerular means are unknown. Plasma protein binding is reported to be independent of the ofloxacin concentration; it was reported to be 25% in healthy volunteers (Lode *et al.* 1987) and was estimated as 32% from *in vitro* data (Janssen-Ortho 2006).

The WHO suggests a drug susceptibility testing critical concentration for ofloxacin of 2.0 mg/L for both solid and liquid media (WHO 2008). Patients with MDR-TB strains with an ofloxacin MIC greater than 2.0 mg/L should not receive ofloxacin as part of their treatment, but should receive an alternative drug. The MIC of ofloxacin from clinical *M. tuberculosis* isolates has been reported to be normally distributed, ranging between 0.25 and

1 mg/L (Angeby *et al.* 2010). However, these data were from a Swedish hospital, and South African MIC distributions may differ.

*In vitro*, murine (Schentag *et al.* 2003) and clinical studies of fluoroquinolones showed they had greatest bactericidal activity against *M. tuberculosis* and decreased probability of resistance when the free (f) AUC/MIC ratio is  $\geq 100$  (Ginsburg *et al.* 2003; Schentag *et al.* 2003; Schentag *et al.* 2003; Gumbo *et al.* 2004; Shandil *et al.* 2007).

The pharmacokinetics and pharmacodynamics of ofloxacin in patients with MDR-TB in a routine clinical setting have been studied to a very limited extent (Stambaugh *et al.* 2002) and have not been studied in South Africans. This study characterises ofloxacin pharmacokinetics and pharmacodynamics in patients with MDR-TB in the high-burden South African setting. The objective was to describe ofloxacin pharmacokinetics in South African patients being treated for MDR-TB, and assess the adequacy of ofloxacin drug exposure with respect to the probability of pharmacodynamic target attainment (fAUC/MIC ratio of at least 100).

## **9.2 Methods**

The group of study participants were being treated for MDR-TB at 2 hospitals located in Cape Town and Durban as outlined in the methods section 4.3. The patient characteristics are reported in table 4.3.

### **9.2.1 Pharmacokinetic analysis**

Using NONMEM, various structural models were evaluated including a one and two compartment model with first order elimination and mixed order elimination. Absorption

models that were evaluated include first order absorption, zero order absorption, sequential zero order and first order absorption and a transit compartment (Savic *et al.* 2007) absorption model. Covariates that were investigated included total body weight introduced using allometric scaling (Anderson and Holford 2008), lean body weight, LBW (Janmahasatian *et al.* 2005) introduced using allometric scaling, creatinine clearance, HIV infection and meal administration (with a meal for patients in Cape Town, and without a meal for patients in Durban). Creatinine clearance in mL/min was calculated using the standard Cockcroft Gault equation as explained in section 4.3.4. In addition, creatinine clearance was also calculated using lean body weight in a modified Cockcroft Gault equation as shown below:

$$\text{Creatinine Clearance} = \frac{(140 - \text{age}) * \text{LBW}}{\text{serum creatinine}}$$

The 2 values of creatinine clearance based on the different calculation methods were both tested separately as covariates in the model.

As the two different study sites had different pharmacokinetic sampling schedules, this had the potential to confound analyses when investigating the effect of food on drug administration. To evaluate the influence of pharmacokinetic sampling differences on the estimates of the model parameters of interest to be evaluated, a stochastic simulation-estimation experiment of 200 samples was undertaken using the Durban absorption model parameter estimates, but using the Cape Town (sparse) pharmacokinetic sampling schedule. The bias and precision of the parameters from the simulation-estimation experiment were obtained. It must however be stated that another potential confounder is the study site, since only Cape Town patients received food with their tablets. This can however not be resolved by any means, and it is more likely that any potential differences would be due to food effects.



### 9.2.2 Probability of target attainment

The probability, based on Monte Carlo simulations, that a specific value of a pharmacodynamic index is achieved or exceeded within a population of individuals is known as the probability of target attainment (PTA) (Mouton *et al.* 2005). The PTA is based upon integration of pharmacokinetic data in humans using Monte Carlo simulations, with antimicrobial pharmacodynamics as has been performed previously (Drusano *et al.* 2001; Preston *et al.* 2003). In our case, the pharmacodynamic index target was a fAUC/MIC ratio of at least 100. Some authors have also used a minimum target fAUC/MIC ratio of 40 (Peloquin *et al.* 2008) hence the following analyses were repeated for this lower target as well. Individual AUCs from the model were obtained by integrating drug concentration predictions from 0-24 hours after drug administration. The AUC was then multiplied by 0.75 which is the unbound fraction of ofloxacin in humans (Lode *et al.* 1987) to obtain the fAUC. The final model was used to perform Monte Carlo simulations in 10,000 individuals to determine the PTA at various MICs (0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L) and the PTA expectation based on the MIC distribution in our study population. The PTA expectation was calculated using the following equation:

$$\sum_{i=1}^n PTA_i \times F_i \quad (\text{Mouton } et \text{ al. } 2005)$$

where  $PTA_i$  is the PTA for each MIC category and  $F_i$  is the fraction of the study population for the corresponding MIC category.

### 9.2.3 Dosing simulations

To optimise the dosing, further Monte Carlo simulations were performed using higher ofloxacin doses ranging from 800 mg to 1600 mg daily in 200 mg increments towards dose optimization that would lead to higher PTA expectation values. The final pharmacokinetic

model implemented in the software NONMEM was used for the simulations. The simulations were carried out in 10,000 patients based on the covariate distribution of our current dataset. For each dose, the fAUC for each patient was obtained. This was then used to calculate the PTA for each MIC in the observed range (0.25-8 mg/L), followed by the PTA expectation as described above.

## 9.3 Results

### 9.3.1 MIC distributions

MIC data were available from 22 of the Durban patients and all 38 of the Cape Town patients. Five of the Durban patients did not have MIC results due to lack of a *M. tuberculosis* isolate from these patients. Table 9.1 below shows the percentages of the MICs for each study site, together with the overall MIC percentages of the pooled data.

Table 9.1: Comparison of MICs for ofloxacin of multi-drug resistant *M. tuberculosis* isolates (%) from 2 sites in South Africa

	Ofloxacin MIC in mg/L				
	0.5	1	2	4	8
Cape Town (n=38)	5	50	32	3	10
Durban (n=22)	41	50	5	0	5
Total (n=60)	18	50	22	2	8

Cape Town MICs were significantly higher than the Durban MICs by approximately one dilution. The geometric means (95% confidence intervals) for the Cape Town and Durban MICs respectively were 1.5 (1.2-2.0) and 0.8 (0.6-1.1), (Wilcoxon rank-sum  $p < 0.001$ ).

### 9.3.2 Ofloxacin pharmacokinetics

An outline of the model building process for ofloxacin is shown in table 9.2 below.

Table 9.2: Summary table of ofloxacin population model development

Model	Description	OFV	Comment
1	One compartment model, first order absorption and first order elimination. Variability on CL, V, KA and F	859	Variability in V went towards zero and was removed with no change in OFV
2	2 compartment model from model 1	859	Not significant
3	Add lag time to model 1, but retaining variability on V	739	Variability in V now estimated to be 25%
4	Test saturable absorption on model 3	734	Small Km value of 8 mg suggests zero order absorption
5	Test mixed order elimination on model 3	739	Both Vmax and Km went up to 10 <sup>7</sup> suggesting first order elimination only
6	Zero order absorption model from model 3. Variability on duration and KA	710	
7	Put covariance between duration and KA	710	Not significant hence excluded
8	2 compartment model from model 6	688	2 compartment model now appears with correct absorption model
9	Test mixed order elimination on model 8	682	No difference in diagnostics coupled with small OFV change so not included
10	Test parallel first order and mixed order elimination	680	No significant difference, hence proceed with first order elimination
11	Sequential zero order and first order absorption on model 8	678	Proceed with this model
12	Site specific zero order duration and KA from model 11	656	Faster absorption in Durban patients than in Cape Town patients
13	Dual clearance with CrCl (calculated using normal Cockcroft-Gault equation) covariate on 1 CL whilst the second CL had no covariate. From model 12	647	Lower OFV
14	Replaced CrCL with value obtained using modified Cockcroft-Gault equation with LBW	641	Hence proceeded with LBW in CrCl calculation
15	To model 14, add allometric scaling of weight on the second CL, central and peripheral volume, and intercompartmental clearance	624	
16	Replaced variability in F with	621	Enabled estimation of variability in

	covariance between the random effects of CL and V		V which had gone towards zero once again
17	Sex covariate on central volume from model 16	609	Females (some of whom were obese) had 70% higher volume than males
18	Used LBW instead of bodyweight as covariate on central volume	600	Effect of sex on central volume no longer significant
19	Replaced sequential zero and first order absorption with transit compartment absorption model and site-specific MTT from model 18	591	Final model. Same number of parameters, as KA was made equal to KTR (transit rate constant)

A transit compartment model best described the absorption of ofloxacin, whilst a two compartment model with first order elimination best described the disposition of ofloxacin. Mixed order elimination could not be supported by the data. As can be seen from the derived parameters in table 9.3 and figure 9.2, there is a short early distribution phase, followed by a longer terminal phase, similar to previous reports for ofloxacin (Lode *et al.* 1987). The final model had two clearance pathways according to the equations below:

$$CL_{GFR} = \theta_1 \times \left( \frac{CrCl}{68} \right)$$

$$CL_{non-GFR} = \theta_2 \times \left( \frac{WT}{70} \right)^{\frac{3}{4}}$$

$$(CL/F)_i = [CL_{GFR} + CL_{non-GFR}] \cdot \exp(\eta_{CL})$$

where  $CL_{GFR}$  is the glomerular filtration of ofloxacin,  $\theta_1$  is the typical value that will be estimated by the model,  $CrCl$  is the creatinine clearance (calculated using the modified Cockcroft-Gault equation) of the individual subject  $i$ , which had a median value of 68 mL/min,  $CL_{non-GFR}$  is the extra-glomerular excretion route of ofloxacin,  $\theta_2$  is the typical value that will be estimated, WT is the weight of individual  $i$ ,  $(CL/F)_i$  is the total oral clearance of ofloxacin for individual  $i$ ,  $\eta_{CL}$  is the population variability in oral clearance for individual  $i$ .

The excretion of ofloxacin was best described using two clearance pathways. One pathway represents glomerular filtration of ofloxacin. Creatinine clearance was a significant covariate for this route of excretion. Substitution of LBW (Janmahasatian *et al.* 2005) (which had a median value of 46 kg) for total body weight in the Cockcroft-Gault equation further improved the model fit and resulted in a further 6 point decrease in the OFV ( $p < 0.01$ ) compared to a model using total body weight. The second excretion route of ofloxacin in our model represents extra-glomerular routes, which would be mainly active tubular secretion and a small amount of biliary excretion (Janssen-Ortho 2006). A significant covariate on this extra-glomerular excretion of ofloxacin was total body weight, introduced allometrically (Anderson and Holford 2008). LBW was investigated as a covariate instead of total body weight, on the extra-glomerular route of excretion, but the addition of LBW resulted in a higher OFV than using total body weight. The central volume of distribution was allometrically scaled to LBW, whilst the peripheral volume was scaled to total body weight, as was the intercompartmental clearance. The central volume of distribution was initially scaled to the total body weight. This resulted in a typical female having a volume of distribution 70 % higher than that for a male of the same weight, and was accompanied by a 12 point drop in the OFV. However, when central volume was scaled to LBW in the final model which described the data equally well, the sex effect on volume fell to 21 % and was no longer statistically significant. HIV infection was not a significant covariate on ofloxacin pharmacokinetics. Administration of ofloxacin after a meal (Cape Town patients) resulted in a 2.4 fold increase in the MTT, meaning food significantly delays the rate of absorption. Population parameter estimates, variability and precision from the final model are found provided in table 9.3. The final control stream is shown in appendix 3.5.

Table 9.3: Parameter estimates from final model, ofloxacin pharmacokinetic study in patients with MDR TB, South Africa

Estimated Parameters	Estimate (% RSE <sup>§</sup> )	BSV (% RSE)
Glomerular filtration L/h/ 68 mL/min CrCl.	3.7 (30)	26 (9)*
Extra glomerular excretion L/h/70 kg.....	4.7 (28)	26 (9)*
Central volume L/46 kg LBW.....	52 (20)	30 (32)
Peripheral volume L/70 kg.....	40 (25)	-
Intercompartmental clearance L/h/70 kg.....	59 (44)	-
Durban mean transit time h.....	0.74 (18)	54 (14)
Cape Town mean transit time h.....	1.76 (11)	54 (14)
Number of absorption transit compartments.....	6 (15)	-
Additive error mg/L.....	0.6 (6)	-
Proportional error %.....	9.6 (9.4)	-
Covariance between random effects of clearance and central volume of distribution	0.56 (25)	-
<u>Derived parameters</u>	<u>Typical Value</u>	
Alpha half life h	0.3	-
Beta half life h	7.8	-
$Kel_{\alpha} h^{-1} §$	2.7	-
$Kel_{\beta} h^{-1} §$	0.09	-
Cmax in Durban patients mg/L	10.4	-
Cmax in Cape Town patients mg/L	8.8	-
Time to Cmax in Durban patients h	1.2	-
Time to Cmax in Cape Town patients h	3	-

<sup>§</sup>  $Kel_{\alpha}$  – elimination rate constant for the alpha phase,  $Kel_{\beta}$  - elimination rate constant for the beta phase

\*variability was put on the overall clearance, which was the sum of the two different pathways.

The bottom of figure (9.1) below is a VPC of the final model and it illustrates that the model described the data well for both study sites, compared to that of the base model which had no covariates. This is followed by the typical concentration-time profiles for a Durban patient

and a Cape Town patient, showing the effect of food lowering the C<sub>max</sub> and prolonging the T<sub>max</sub> in Cape Town patients.

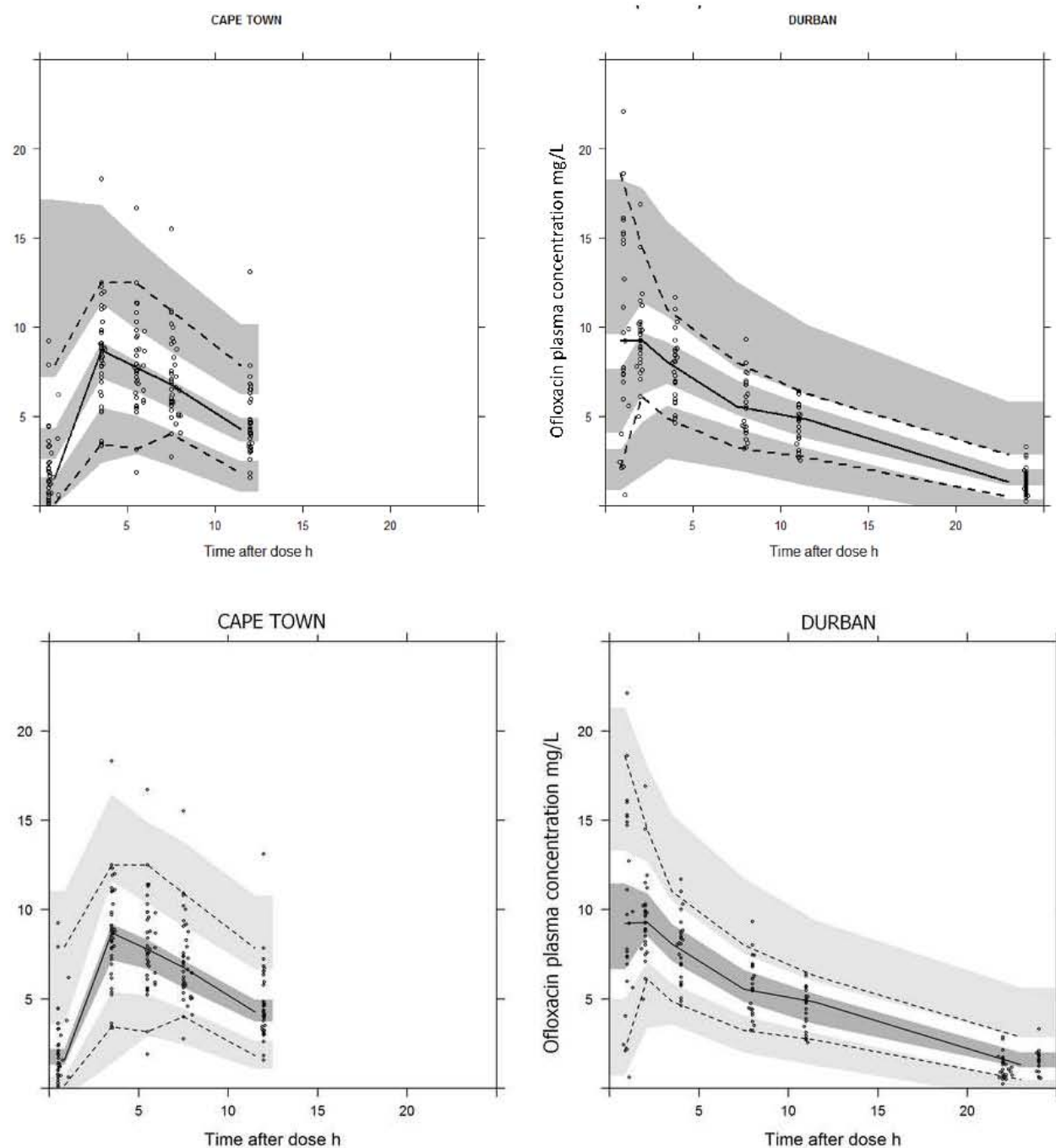


Figure 9.1: VPC of ofloxacin plasma concentrations in South African patients with MDR TB in the final model stratified by study site. Top – Base model (model 11), bottom – Final model (model 19).

The open circles are the observations. The upper dotted line represents the 95<sup>th</sup> percentile of the observations. The continuous line represents the median of the observations. The lower

dotted line represents the 5<sup>th</sup> percentile of the observations. The shaded areas are the simulated confidence intervals for the corresponding percentiles.

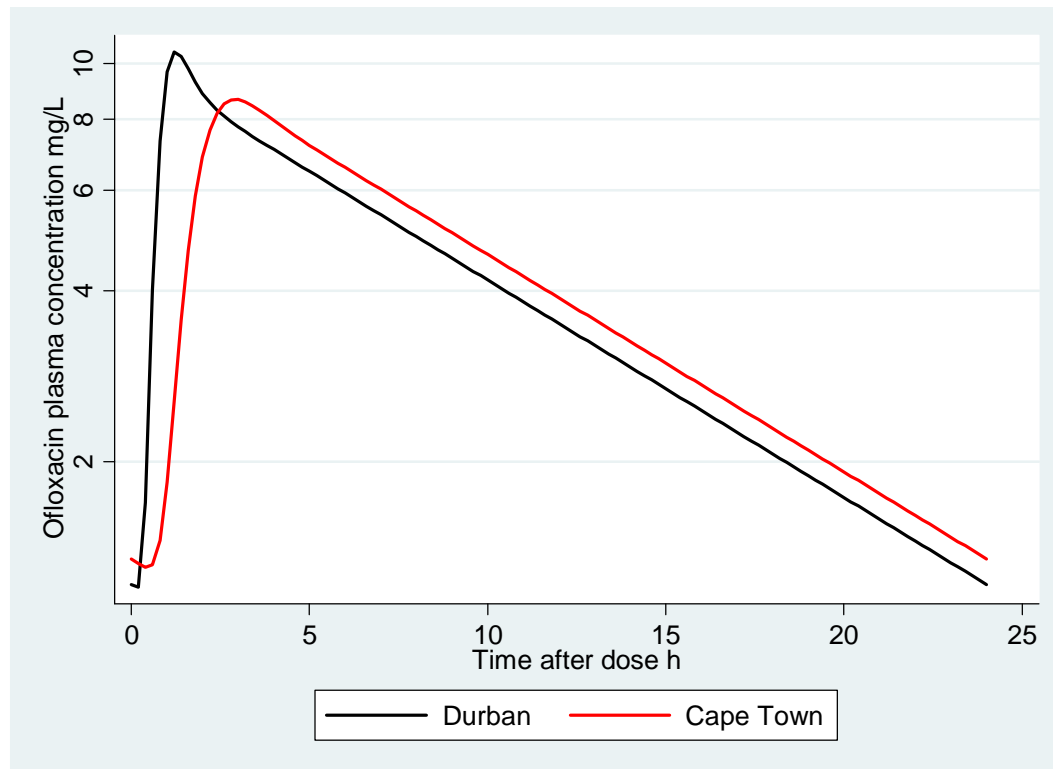


Figure 9.2: Log-normal plot showing ofloxacin concentration-time profile for the typical patient in Cape Town and in Durban

There was no significant difference in  $AUC_{0-24}$  between the Cape Town patients and the Durban patients. The typical  $C_{max}$  for the 2 study sites are found in table 9.2 above. Figures 9.3 and 9.4 below show the distribution of the AUC and  $C_{max}$  stratified by study site.



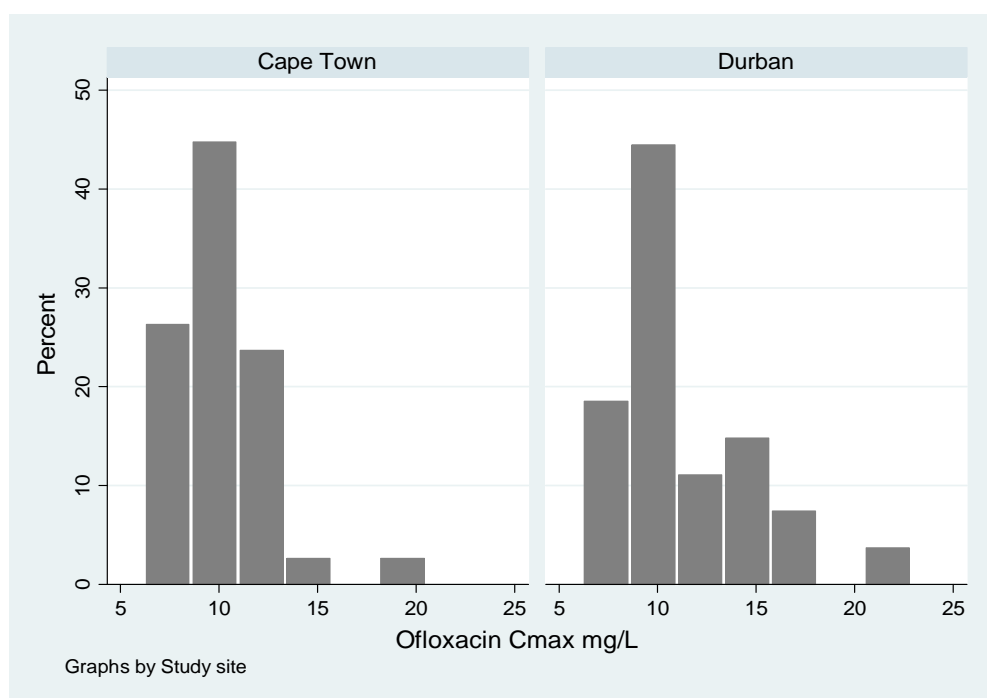


Figure 9.3: Histograms showing distribution of ofloxacin Cmax for Cape Town and Durban patients.

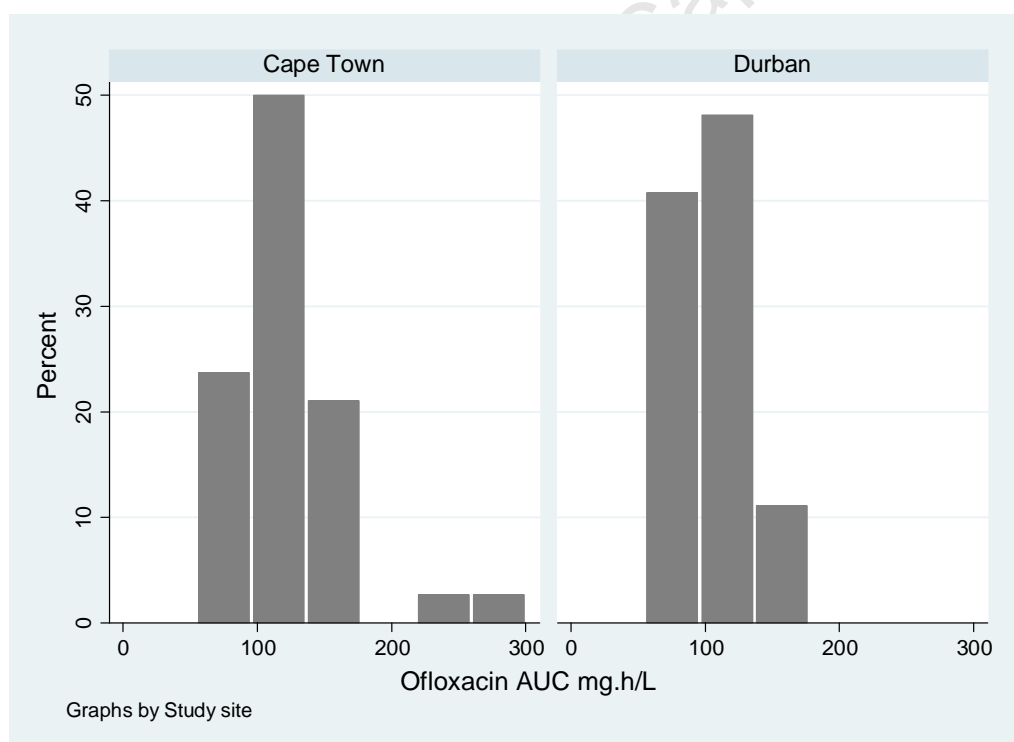


Figure 9.4: Histograms showing distribution of ofloxacin AUC<sub>0-24</sub> for Cape Town and Durban patients.

The median (interquartile range) for the C<sub>max</sub> and AUC were 9.8 (8.9-12.1) mg/L and 105 (85-122) mg.h/L. One patient from Cape Town had a very high ofloxacin AUC of 300 mg.h/L. This patient had a CrCl of just 61 mL/min (and a body weight of 56 kg), hence compromised renal function may have led to such a high exposure. The patient with the second highest exposure of 236 mg.h/L had a normal CrCl of 109 mL/min (and a body weight of 55 kg) therefore other unknown mechanisms may have caused this high exposure.

As earlier stated, a stochastic simulation and estimation experiment was carried out to determine whether this finding could be an artefact of an altered sampling schedule. The bias and precision of the MTT from this were found to be +0.9 % and 13 % respectively, thus confirming that the finding was independent of the study design and sampling schedule.

### 9.3.3 Ofloxacin pharmacodynamics

The graphs of the PTA corresponding to various ofloxacin doses from Monte Carlo simulations are shown below.

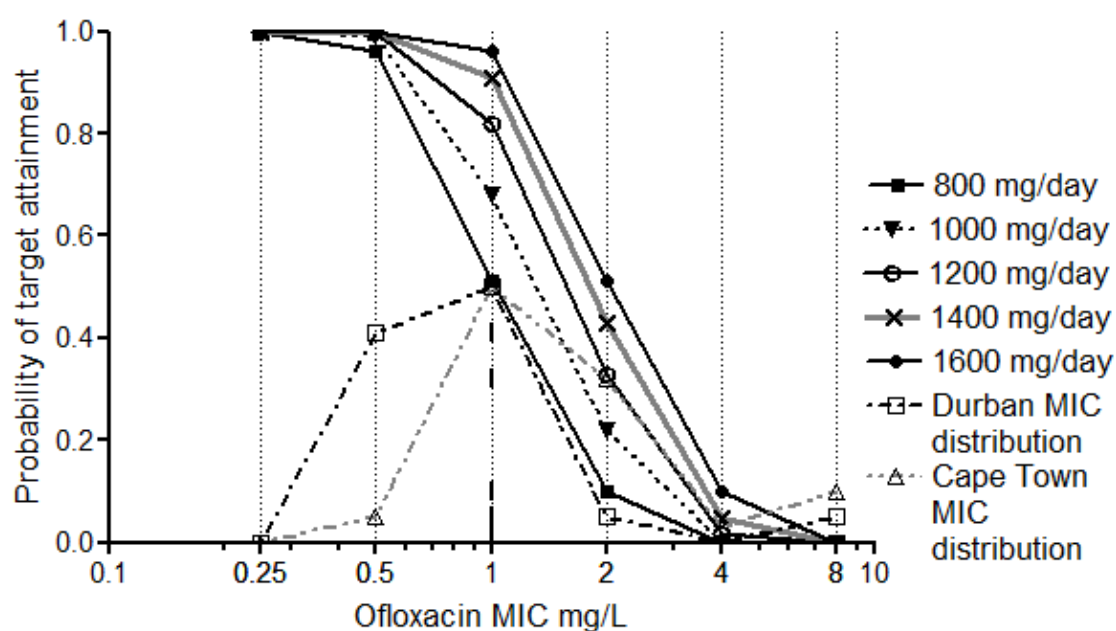


Figure 9.5: Probability of target attainment (fAUC/MIC  $\geq 100$ ) by *M. tuberculosis* isolate minimum inhibitory concentration (MIC) for ofloxacin for various daily doses of ofloxacin. The fraction of the population with each MIC is also shown on the same graph, for both Durban and Cape Town patients.

Since some authors have proposed a minimum target fAUC/MIC ratio of 40 (Peloquin *et al.* 2008), figure 9.6 shows the probability of attaining this target for the current population.

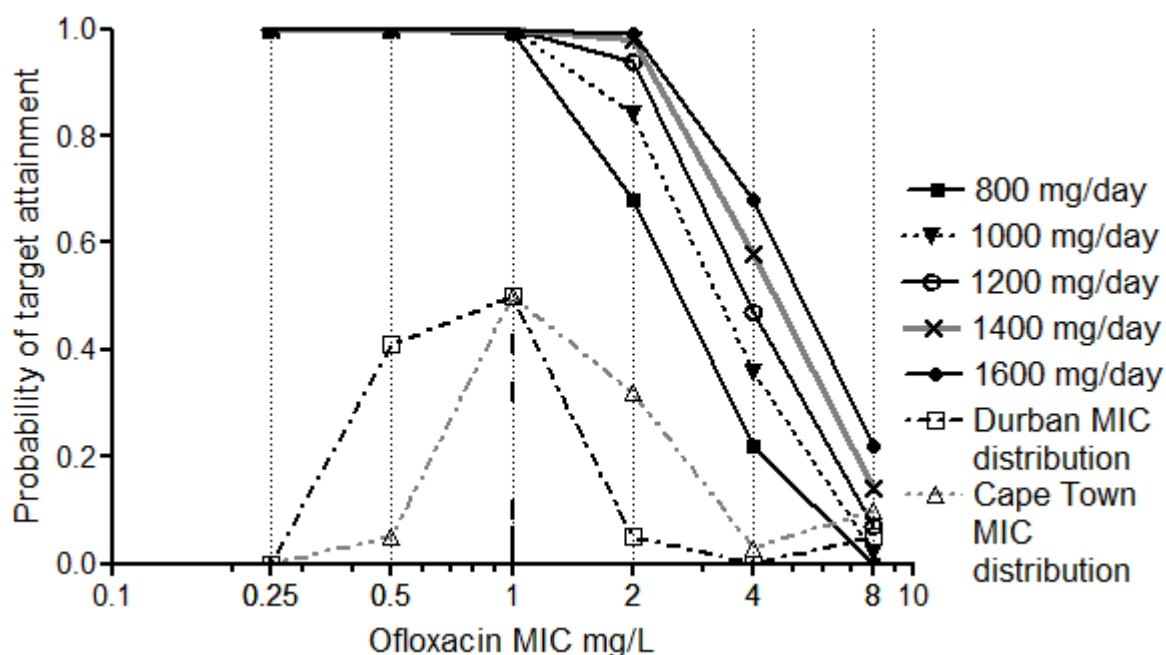


Figure 9.6: Probability of target attainment (fAUC/MIC  $\geq 40$ ) by *M. tuberculosis* isolate MIC for ofloxacin for various daily doses of ofloxacin. The fraction of the population with each MIC is also shown on the same graph, for both Durban and Cape Town patients.

Using the WHO recommended critical concentration of 2.0 mg/L, none of the doses examined (800 mg to 1600 mg) resulted in a PTA greater than 0.9. The 800 mg dose provided a PTA more than 0.9 only for patients with MICs less than or equal to 0.5 mg/L, that is, 5.3% of Cape Town patients and 40.9% of Durban patients. A dose of 1400 mg was the minimum dose achieving a PTA greater than 0.9 in patients with an MIC equal to 1 mg/L. The PTA expectation values for the pooled study population, as well as those for each study site are shown in the tables below.

Table 9.4: PTA expectation values ( $fAUC/MIC \geq 100$ ), ofloxacin pharmacokinetic study in patients with MDR TB, Cape Town and Durban, South Africa

Ofloxacin daily dose mg	Overall PTA expectation	Cape Town PTA expectation	Durban PTA expectation
800	0.45	0.33	0.65
1000	0.57	0.46	0.76
1200	0.66	0.57	0.83
1400	0.73	0.64	0.89
1600	0.77	0.70	0.91

Table 9.5: PTA expectation values ( $fAUC/MIC \geq 40$ ), ofloxacin pharmacokinetic study in patients with MDR TB, Cape Town and Durban, South Africa

Ofloxacin daily dose mg	Overall PTA expectation	Cape Town PTA expectation	Durban PTA expectation
800	0.83	0.77	0.94
1000	0.87	0.83	0.95
1200	0.90	0.87	0.96
1400	0.92	0.89	0.97
1600	0.93	0.91	0.97

The simulations showed that an ofloxacin daily dose of at least 1600 mg would achieve a PTA ( $fAUC/MIC \geq 100$ ) expectation value higher than 0.9 in the Durban population where the MICs were significantly lower than for the Cape Town cohort.

## 9.4 Discussion

This is the first report describing the pharmacokinetics and pharmacodynamics of ofloxacin taking into account *M. tuberculosis* susceptibility data from MICs in the South African study population. A high proportion of South African patients fail to achieve the target fAUC/MIC of 100. Significantly higher MICs to ofloxacin were observed for isolates from Cape Town (in the Western Cape Province in South Africa) than from Durban (in Kwa-Zulu Natal Province in South Africa). The MICs were determined in the same laboratory. Due to the limited sample size these data may not represent the general epidemiological situation therefore further study of ofloxacin MICs in South Africa is warranted. However, resistance of the W-Beijing strain to fluoroquinolones has been documented (Duong *et al.* 2009), and the W-Beijing strain is rapidly increasing in the Cape Town (Cowley *et al.* 2008). It is however difficult to attribute the higher MICs found in Cape Town to the W-Beijing strain, since Durban has a high prevalence of the F15/LAM4/KZN strain (Pillay and Sturm 2007) which is also resistant to fluoroquinolones. Further studies to determine the prevalence of different types of strains across South Africa, and their susceptibilities to different drugs are needed.

This is also the first report to quantitatively describe ofloxacin excretion by glomerular and extra-glomerular means, together with the covariates influencing the different pathways. However, these findings should be interpreted with caution since no direct intra-renal drug sampling was performed and the results are derived solely from a mathematical perspective. The finding that calculation of glomerular filtration using LBW rather than total body weight described the glomerular clearance better is in agreement with the fact that renal function is more closely related to lean body weight (Janmahasatian *et al.* 2008). This finding is especially important in our setting where some of the patients are obese according to their

high body mass indices. Although some papers indicate that hyperfiltration may occur in obesity (Chagnac *et al.* 2008), more recent papers show that using the Cockcroft and Gault formula with an adjustment for LBW is the best measure for renal function in obesity (Jesudason and Clifton 2011). From the current model, it can be seen that patients with renal insufficiency or patients with lower body weight would have lower clearance of the drug, and hence higher plasma concentrations. When considering administration of higher doses of ofloxacin it may be important to consider individual variation in body weight and renal function. HIV infection did not significantly alter ofloxacin pharmacokinetics in accord with previous reports (Stambaugh *et al.* 2002).

The simulations of higher doses are based on first order elimination of ofloxacin. Mixed order elimination of the drug in the study population was investigated, but this was not found to be statistically significant at daily doses of 800 mg. Hence the model would under-predict exposure at higher doses, if saturable kinetics occurs.

With an MIC of 2.0 mg/L, (the WHO critical concentration), none of the doses simulated would result in an acceptable PTA, suggesting that the critical concentration should be revised downwards. Indeed, other authors have proposed a cut-off of  $\leq 1.0$  mg/L based upon the MIC distribution from clinical strains in Sweden (Angeby *et al.* 2010). The PTA expectation results support this argument. This is apparent even though the MIC determination method in our study differs slightly from the Swedish study which defined the MIC as lowest concentration of drug that inhibited >99% of the bacterial population. This would result in a PTA greater than 0.9 but only when using a daily dose of at least 1400 mg. The safety of these higher ofloxacin doses needs evaluation as fluoroquinolones have side effects such as dysglycaemia, tendonitis, anaemia (Janssen-Ortho 2006), and QT interval

prolongation, (Falagas *et al.* 2007) whose incidence and severity may increase with higher doses. This is especially of concern when one considers the long duration of MDR-TB treatment. For the current 800 mg daily dose, we propose an MIC cut-off of 0.5 mg/L as can be seen from the figure with the PTA plots. However, if a target fAUC/MIC ratio of 40 is used, a breakpoint of 1 mg/L is recommended as can be seen from figure 9.6. In either case, ofloxacin should not be used in patients with an MIC >1 mg/L. Therefore one can speculate that the way ofloxacin is currently being used might even be promoting resistance among fluoroquinolones since it is known that there is cross-resistance amongst the fluoroquinolones (Ginsburg *et al.* 2003; Sirgel *et al.* 2012).

Perhaps ofloxacin continues to be used because it is relatively inexpensive. However, it is unclear whether this is rational drug use since our data suggest otherwise. The levorotatory isomer of ofloxacin, levofloxacin, has been found to have half the MIC of ofloxacin against *M. tuberculosis* (Hu *et al.* 2003) therefore it may be expected to be more potent than ofloxacin. Indeed, in mice it has been shown that double the ofloxacin dose is equivalent in antitubercular activity to the corresponding levofloxacin dose (Ji *et al.* 1995). Hence one can expect a similar dose of levofloxacin to do much better than ofloxacin. However, the higher cost of levofloxacin remains a problem. The same goes for moxifloxacin. Ofloxacin has been found to be less effective compared to moxifloxacin and gatifloxacin in clinical studies (Rustomjee *et al.* 2008). Use of these alternative drugs would significantly increase the PTA expectation in patients with MDR-TB and decrease the likelihood of development of resistance.

The PTA expectation for Durban patients was significantly higher than that for Cape Town patients. Since there was no difference in the  $AUC_{(0-24)}$  between the 2 sites, the PTA

expectation difference is due to the difference in MICs, which were found to be lower in Durban as stated earlier.

#### **9.4.1 Limitations**

A fAUC/MIC ratio of 100 was used as the ideal minimum value based upon studies using animals infected with *M. tuberculosis* (Shandil *et al.* 2007) as well as data from human studies (Schentag *et al.* 2003) and *in vitro* studies (Gumbo *et al.* 2004). There is some disagreement over what the ideal fAUC/MIC ratio should be for fluoroquinolones (Schentag *et al.* 2003) which may vary for different types of bacteria (Wright *et al.* 2000) and during different phases of TB treatment. Concentration independent protein binding of ofloxacin was assumed in accordance with findings in healthy volunteers (Lode *et al.* 1987). However, it is possible that at higher concentrations, ofloxacin protein binding may be concentration dependent as reported in a murine study (Shandil *et al.* 2007). Furthermore, a fixed value of 25% may be inaccurate in some patients since they may differ from each other.

#### **9.5 Conclusion**

The results suggest that the currently recommended ofloxacin dose of 800 mg per day is too low for the treatment of MDR-TB in South Africa. If higher doses of ofloxacin cannot be used due to safety reasons, a more potent fluoroquinolone such as levofloxacin or moxifloxacin should be used.



## **10. A NOVEL TIME TO EVENT PHARMACODYNAMIC MODEL DESCRIBING TREATMENT RESPONSE IN PATIENTS WITH PULMONARY TUBERCULOSIS USING DAYS TO POSITIVITY IN AUTOMATED LIQUID MYCOBACTERIAL CULTURE**

*Antimicrobial Agents and Chemotherapy*. 2013 Feb;57(2):789-95

### **10.1 Introduction**

There is a need for a method that quantitatively describes the change in days to positivity in automated liquid culture over time in patients receiving antituberculosis treatment. The aim of this work was to describe the decline in viable mycobacteria in the sputum during the 8 week intensive phase of standard short course chemotherapy in patients with pulmonary TB using the quantitative measure of days to positivity in liquid culture by developing a NLME repeated time to event modeling approach. A model that maximizes the use of the quantitative nature of MGIT days to positivity results to describe the mycobacterial response to treatment over time in sputum samples from patients, combined with the growth kinetics of the mycobacteria, could be useful for quantitatively analysing similar data from studies investigating new drug combinations or testing the effect of various covariates on disease regression in patients.

### **10.2 Methods**

Information relating to the recruitment of study participants and their characteristics can be found in the general methods sections 4.1 and 4.2.

### 10.2.1 Data Analysis

Population parameter estimates and variability for a repeated time to event model were obtained using the Laplacian estimation method in NONMEM, which has been shown to have low parameter bias and imprecision when a high proportion of individuals in the dataset have events (Karlsson *et al.* 2011). A bootstrap of 500 replicates was carried out on the final model to obtain estimates of parameter precision.

For the repeated time to event model, a positive MGIT culture result was treated as an event, whilst a negative culture result was treated as a right-censored observation. Mono-, bi- and tri-exponential models were investigated in an attempt to describe the decline in bacillary load in the weekly sputum specimens in patients. The relative amount of bacteria in the bacterial growth compartment in the model (corresponding to the MGIT inoculum) was initialized to the value estimated from these models. The term relative amount is used because the amounts do not relate to actual numbers of bacteria, but upon integration according to the growth models below, are an equivalent related to oxygen consumption which is then directly related to the hazard of a positive test result. In other words, after the subsequent mathematical calculations and integration, the relative amount must translate to a reasonable hazard (or probability of obtaining a positive test result). Therefore the relative amount in the MGIT inoculum for a particular week is driven by hazard for that week. Therefore, had true amounts been measured (which would be higher by several orders of magnitude) they would need to be divided by a very large factor to translate to a reasonable probability which would not always be 1. An exponential growth model, the Gompertz model (Gompertz 1825) and the Logistic model (Zwietering *et al.* 1990; Annadurai *et al.* 2000) were investigated to describe the subsequent growth of the mycobacteria in the MGIT culture

during the days of incubation. The differential equations for these models implemented in the ADVAN6 subroutine of NONMEM<sup>®</sup> are shown below:

$$N'_t = N_t \times K_{growth} \quad : \text{Exponential growth model}$$

$$N'_t = N_t \times K_{growth} \times \log(N_{max}/N_t) \quad : \text{Gompertz growth model}$$

$$N'_t = N_t \times K_{growth} \times (N_{max} - N_t) \quad : \text{Logistic growth model}$$

where  $N_t$  is the amount of bacteria in the MGIT culture at time  $t$  after start of incubation,  $N_{max}$  is the carrying capacity of the MGIT culture, and  $K_{growth}$  is a constant relating to the growth rate of the bacteria. Thus at the beginning of the MGIT incubation where  $t$  is zero,  $N_t$  was initialized as described above. The bacteria would then grow until a positive MGIT result was recorded or a negative result after 42 days. The bacterial growth models were investigated with and without a lag phase of the mycobacteria before growth commenced. The lag time in days ( $L$ ) was estimated as a parameter in the model which delayed the commencement of bacterial growth by a period of time ( $L$  days) after the system starts running at time 0, such that bacterial growth commenced at time  $0 + L$  days. An attempt was made to model different growth rates and different lag times of actively replicating bacteria and the slowly replicating dormant bacilli in the MGIT culture.

The hazard of a positive MGIT result was directly related to the cumulative amount of bacteria in the tube as shown below:

$$h(t)dt = N_t = Pr(t \leq T < (t + dt) | T > t)$$

where  $Pr$  is the probability of having an event within the very short time interval  $dt$ , provided one did not have an event before time  $t$ .

The probability of not having a positive sputum MGIT culture event was a function of the cumulative hazard of a positive event (integral of the hazard with respect to time) for each day of incubation in the MGIT culture using the equation below:

$$S(t) = e^{-\int_{t_j}^{t_{j+1}} h(t)dt}$$

The probability density function (pdf) i.e. the likelihood of having an event at time  $t$  when an individual had a positive sputum culture event was calculated as follows:

$$pdf = h(t) \times S(t)$$

Thus when an individual had a positive test result, the pdf was estimated, whereas for negative results, the survival to 42 days was estimated.

Although the primary aim of the work was to develop a method utilizing ‘days to positivity’ data to describe treatment response in patients with TB, the effect of covariates including presence of lung cavitation and HIV infection on disease progression were investigated. For the patients who did not have lung cavitation data available, a mixture model was used to assign an individual to one group or another. The proportion of patients who had no cavitation was fixed to that calculated from those who did have cavitation results.

### 10.3 Results

Ten of the 154 patients recruited in the study were omitted from the analysis because their baseline MGIT culture was negative. Sixteen of the remaining 144 patients (11%) were HIV infected. Ninety-seven (67%) patients had lung cavities visible on chest x-ray, 20 (14%) did not have lung cavitation, and 27 (19%) patients had no available lung cavitation data. Baseline drug susceptibility testing results were available for 118 participants (82%). Of these, 6 (5%) patients had isoniazid monoresistance, 1 (0.1%) had rifampicin monoresistance,

whilst resistance to both rifampicin and isoniazid was found in 4 (3%) patients. Therefore the vast majority of patients had drug susceptible TB. Only 53% of patients had a negative sputum culture result after 8 weeks of treatment. As patients were only followed up to 8 weeks after treatment initiation, there is no further data on the final patient outcomes upon completion of the additional 4 month continuation phase. Figure 10.1 below shows the progression of days to a positive MGIT result with weeks on treatment. It appears as though there is increasing variability with increased study duration.

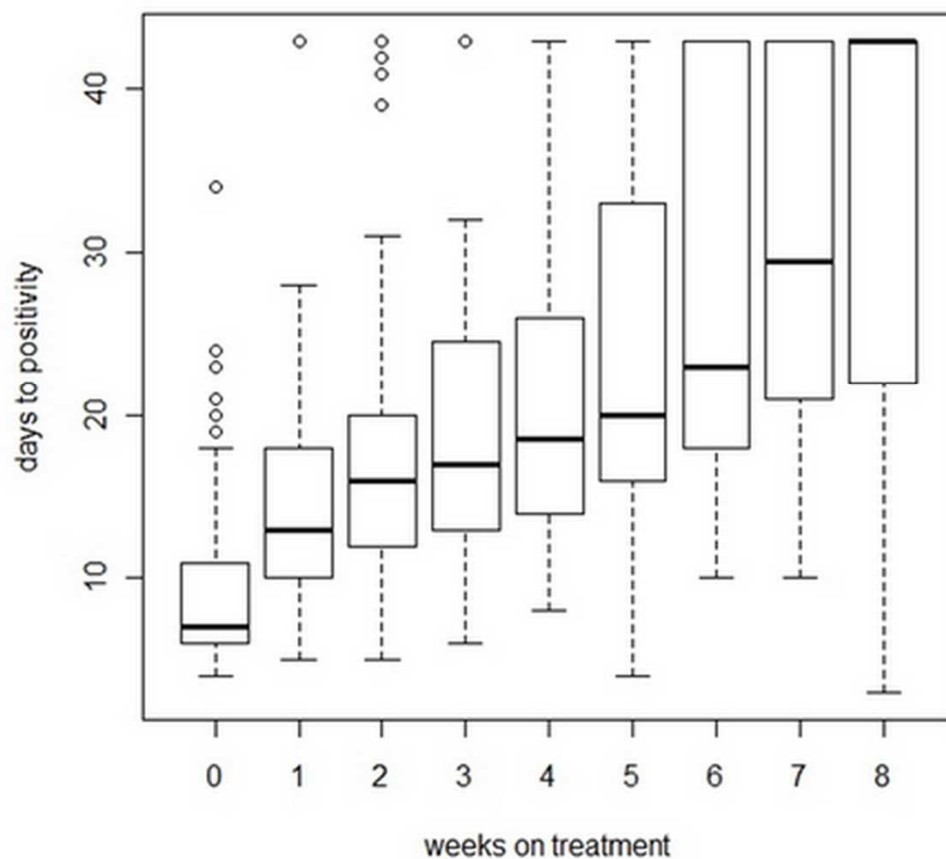


Figure 10.1: Box and whisker plots of days to positivity for each week of treatment

Table 10.1 shows the increasing percentages of patients with negative MGIT culture results as treatment progressed, as well as the percentages of patients with no MGIT culture results at each week, mainly due to contamination of the culture.

Table 10.1: Percentages of patients (N=144) with negative sputum culture results and with missing culture results for each week of treatment for TB

Week of treatment	% negative	% missing data
0	0	4
1	7	5
2	6	3
3	13	5
4	15	6
5	19	9
6	29	11
7	33	13
8	48	10

The model building process involved developing a model for decline in bacillary load in patients, as well as modeling the growth kinetics of the bacteria processed from the sputum specimen in the MGIT culture for the weekly specimens. The model building process is summarized in the table below.

Table 10.2: Summary table of population model development for time to event model using days to positivity data

Model	Description	OFV	Comment
1*	Monoexponential model of bacillary decline in patients: $N_p = N_{p0} \cdot e^{-k \cdot t}$ . Variability on $N_p$ . Followed by exponential growth model of bacteria in MGIT culture.	6665	
2	Biexponential model of bacillary	6636	

	decline in patients from model 1		
3	Triexponential model of bacillary decline in patients from model 2	6625	Two additional parameters and overlapping confidence intervals with biexponential model, hence proceeded with biexponential model
4	To model 2, add lag time of bacteria in MGIT culture before growth commences	6326	
5	From model 4, try Gompertz growth model instead of exponential growth in MGIT culture	6503	Higher OFV. Unreasonable parameter estimates, e.g. early bacterial kill rate of $7 \times 10^{11} \text{ week}^{-1}$
6	From model 4, try logistic growth model instead of exponential growth in MGIT culture	6284	42 point decrease in OFV, hence logistic growth model is significant improvement. However, similar to previous models are very high early kill rates, greater than $10 \text{ week}^{-1}$ , which is not physiological
7	To model 6, add function for decrease in viability of the bacteria with time on treatment i.e. decreased ability of the bacteria to grow in culture as time on treatment progresses.	6171	Significant improvement, although still have early kill rate of $11 \text{ week}^{-1}$
8	From model 7, try separating lag times for bacteria that are killed rapidly (alpha phase) from those that are killed slowly (beta)	6169	Not significant. Alpha phase bacteria (high metabolic activity) had lag time of 3 days, whilst beta phase bacteria (slow growing) had lag time of 4 days
9	From model 7, try estimating different baseline growth rates for the bacterial subpopulations in logistic growth model in the MGIT culture	6077	Now obtain reasonable alpha kill rate of $2 \text{ week}^{-1}$ . Bacteria that were killed rapidly (alpha) were estimated to grow 7 times faster in MGIT culture than those that were killed slowly.
10	From model 7, try estimating different rates of decrease in viability of the 2 bacterial subpopulations	5995	Bacteria that were killed rapidly also lost their viability rapidly in MGIT culture. Better model than model 9, hence this was continued with.
11	From model 10, estimate variability on intercept for rapidly killed bacteria, instead of on the total bacterial population in patients	6057	Worse fit
12	From model 10, estimate variability on rate of kill of rapidly killed bacteria, instead of on the total bacterial population in patients	6255	Worse fit
13	From model 10, estimate variability on intercept for slowly killed bacteria, instead of on the total	6209	Worse fit

	bacterial population in patients		
14	From model 10, estimate variability on rate of kill of slowly killed bacteria, instead of on the total bacterial population in patients	6115	Worse fit
15	From model 10, estimate variability on overall decrease in viability of the bacteria in MGIT culture, instead of on the total bacterial population in patients	5981	Better model than having it on the total bacterial population, hence proceed with this model
16	Add variability on total bacterial population to model 15	5981	Not significant
17	Add within subject variability in total bacterial population to model 15. Nine occasions in total (8 weeks of treatment, plus baseline) and estimate same variability between all of them	5983	Not significant. Model also becomes unstable
18	In separate models from model 15, test HIV on baseline intercept of rapidly killed bacteria, baseline intercept of slowly killed bacteria and the 2 different kill rates	~5979	Not significant
19	Test presence of cavitation as covariate on model 15	5974	Final model. A Weibull distribution of event times model did not change the OFV, with the shape parameter not changing from 1.

\* $N_p$  – Amount of bacteria in patients' sputum,  $N_{p0}$  – Amount of bacteria in patients' sputum at baseline,  $k$  – rate constant bacterial kill in patients,  $t$  – time on treatment

### 10.3.1 Model for decline in bacillary load in the sputum specimens from patients

A biexponential model best described the decline in the number of bacteria in the sputum samples over time on treatment. The model was of the form described in the equation below:

$$\text{Amount of bacteria} = A.e^{-\alpha.t} + B.e^{-\beta.t}$$

where: A is the baseline amount of bacteria that are killed rapidly with the rate of kill being equal to alpha ( $\alpha$ ), and B is the baseline amount of bacteria that are killed more slowly with their rate of kill being equal to beta ( $\beta$ ).



A visual predictive check from 100 simulations is shown below and it shows that the model captured the trend of increasing days to a positive result in the data well.

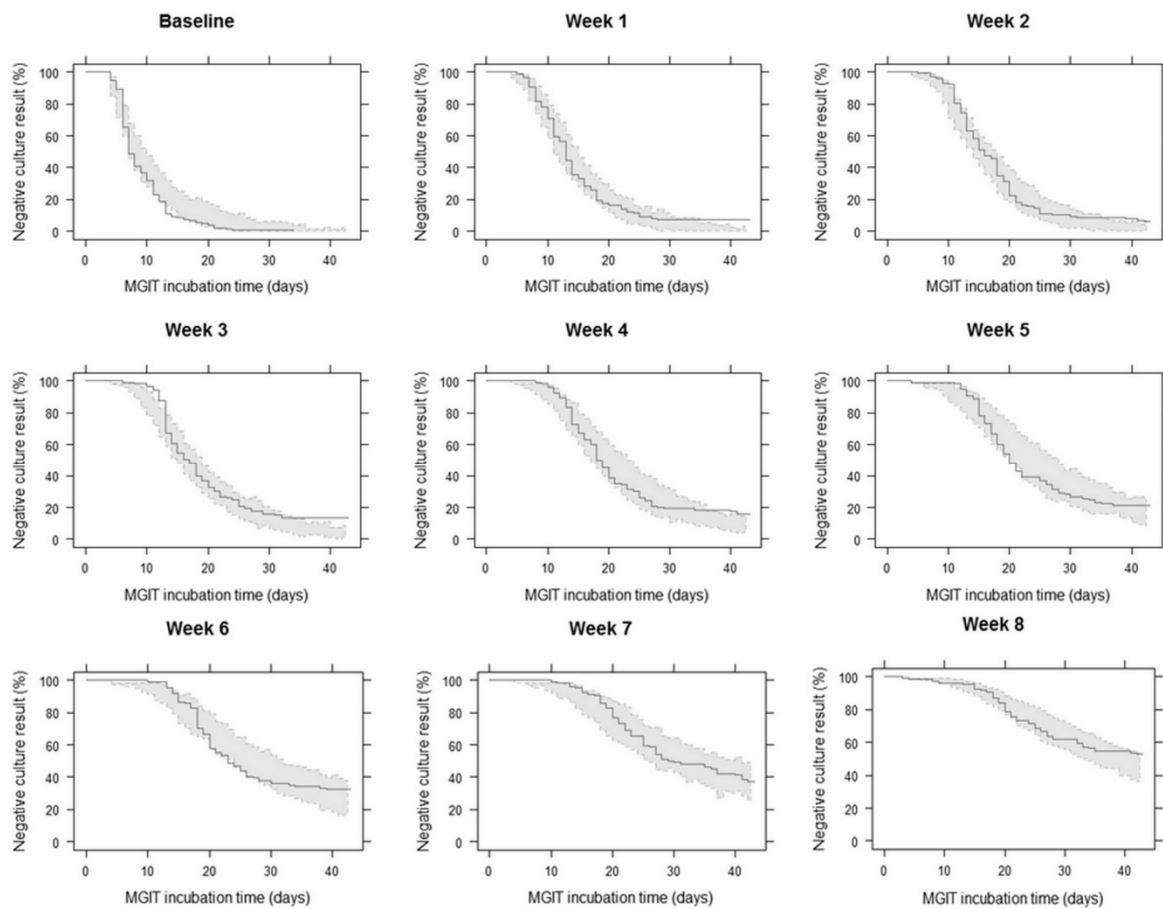


Figure 10.2: A visual predictive check from 100 simulations using the final model stratified into each week of treatment. The continuous line is a Kaplan-Meier plot for the real data. A positive sputum result was regarded as an event and will result in a step on the staircase plot. The shaded area is a 90 % prediction interval based on the simulated data from the model.

Table 10.3 shows the final parameter estimates and their 95 % confidence intervals from a bootstrap of the final model. The final control stream for this model is shown in appendix 3.6

Table 10.3: Population parameter estimates for model describing days to positivity

Parameter	Estimated value (95 % CI)
Baseline amount of rapidly killed bacteria	0.0573 (0.00387, 0.119)
Rate of kill for rapidly killed bacteria ( $\text{week}^{-1}$ )	2.68 (1.07, 4.32)
Baseline amount of slowly killed bacteria	0.00141 (0.0003, 0.00248)
Fractional increase in baseline amount of slowly killed bacteria for presence of lung cavitation	0.728 (0.0782, 4.04)
Rate of kill for slowly killed bacteria ( $\text{week}^{-1}$ )	0.124 (0.0126, 0.173)
Bacterial lag time before growth in MGIT culture commences (days)	4.00 (2.96, 4.00)
Baseline maximum growth rate of bacteria in MGIT culture ( $\text{day}^{-1}$ )	8.31 (4.94, 12.9)
Rate of decrease of maximum growth rate in MGIT culture of rapidly killed bacteria ( $\text{week}^{-1}$ )	0.863 (0.582, 1.83)
Rate of decrease of maximum growth rate in MGIT culture of slowly killed bacteria ( $\text{week}^{-1}$ )	0.283 (0.223, 0.370)
Maximum carrying capacity of MGIT	0.158 (0.133, 0.212)
Population variability in rate of decrease in maximum growth rate of bacteria in MGIT (%)	56.6 (34.1, 71.6)

Deriving from alpha, the typical time to kill half the amount bacteria killed rapidly is 1.8 days. This means that after about 1 week, most of these bacteria are no longer present in the patients' sputum. Similarly for beta, the time to kill half the amount of bacteria killed slowly is 39 days and it can take more than 5 months to kill most of these bacteria. The figure below shows the decline in bacillary burden with time based on the parameter estimates from the final model for a patient with no lung cavitation.

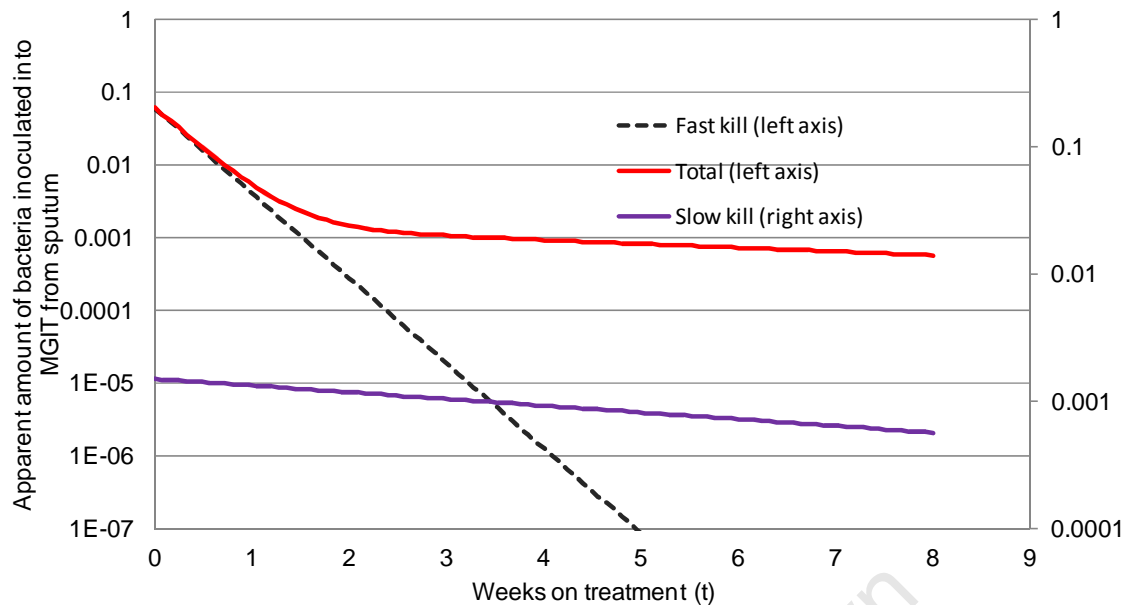


Figure 10.3: Typical relative amount of *M. tuberculosis* processed from patients weekly sputum and inoculated into the MGIT.

At baseline, the model predicts that the more rapidly killed mycobacteria are typically 41 times more prevalent in the MGIT inoculum as calculated from the ratio of baseline amount of rapidly killed bacteria to those that are killed slowly. Patients with lung cavitation present were found to have 1.73 times the bacterial load of patients with no lung cavitation. The ratio of rapidly killed bacteria to slowly killed bacteria at baseline in such patients was 23. HIV infection was not found to have any influence on the decline in bacillary burden in the dataset.

### 10.3.2 Model for growth of mycobacteria in MGIT culture

The amount of bacteria from the patients' sputum was then included in the next part of the model, as a baseline amount of bacteria for inoculation into the MGIT culture. A lag time of 4 days was estimated before bacterial growth in the MGIT culture commenced. The logistic growth model best described the growth of the mycobacteria in the MGIT culture. We also

found that the maximum possible rate of growth decreased from a baseline rate ( $K_{start}$ ) of 8.31 day<sup>-1</sup> as time on treatment progressed according to the equation below:

$$K_{growth(t)} = K_{start} \times e^{-\delta \cdot t}$$

Where  $K_{growth(t)}$  is the maximum possible rate of growth at week (t) and  $\delta$  was a sum of a fractional contribution from bacteria that are killed rapidly and those that were killed slowly as follows:

$$\delta = 0.863 \times \text{Fast Fraction} + 0.283 \times \text{Slow Fraction}$$

From the equation above, the rate of decrease in growth for the bacteria that were killed rapidly was 3 times higher than that for the bacteria that were killed slowly. Population variability in  $\delta$  was estimated using a log-normal distribution. The figure below shows the change of the maximum MGIT growth rate constant with time on treatment.

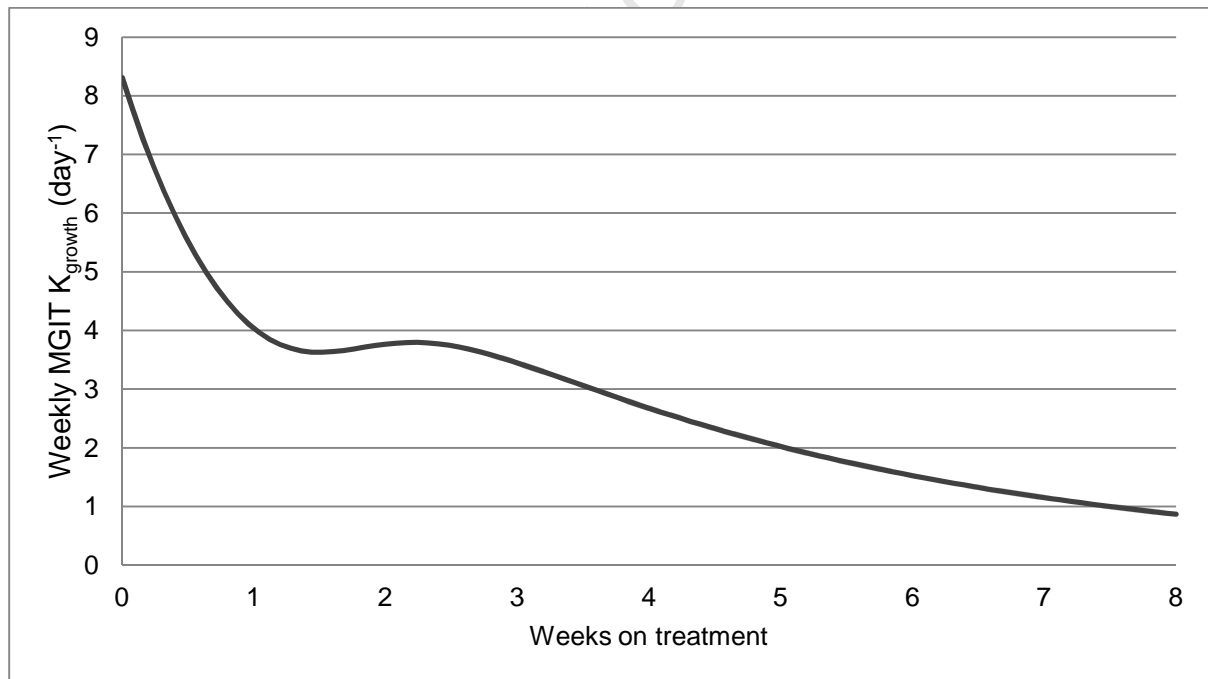


Figure 10.4: Maximum growth rate of mycobacteria in MGIT culture for the logistic growth model as weeks on treatment progress.

### 10.3.3 Survival model

An exponential distribution of the time to obtain a positive MGIT culture result was used in the time to event model, with a Weibull distribution failing to improve the model. The hazard was directly related to the cumulative amount of bacteria in the MGIT culture. The probability of obtaining a positive MGIT culture result up to 42 days of culture for each week of treatment is shown in the figure below.

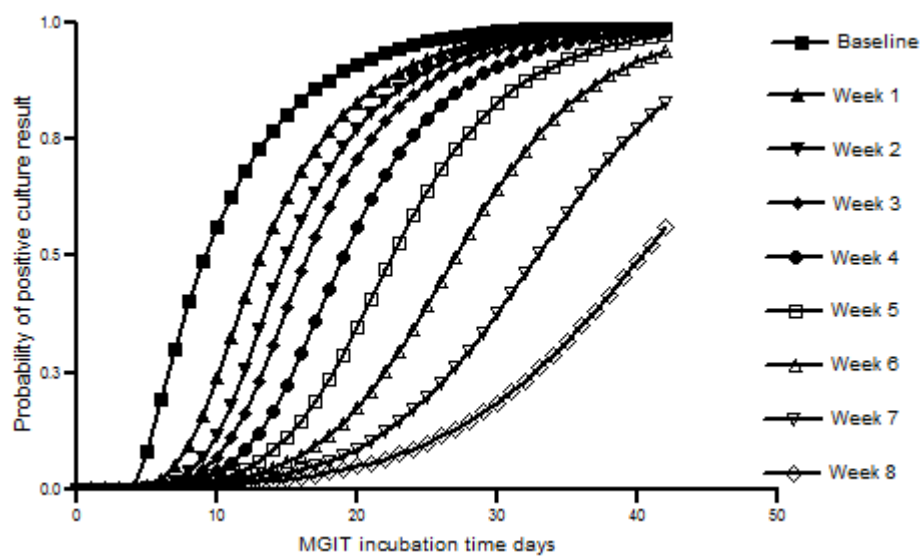


Figure 10.5: Probability of obtaining a positive MGIT culture result upon incubation to 42 days as weeks on treatment progress.

A schematic diagram of the final model summarizing the above is presented in figure 10.6 below.

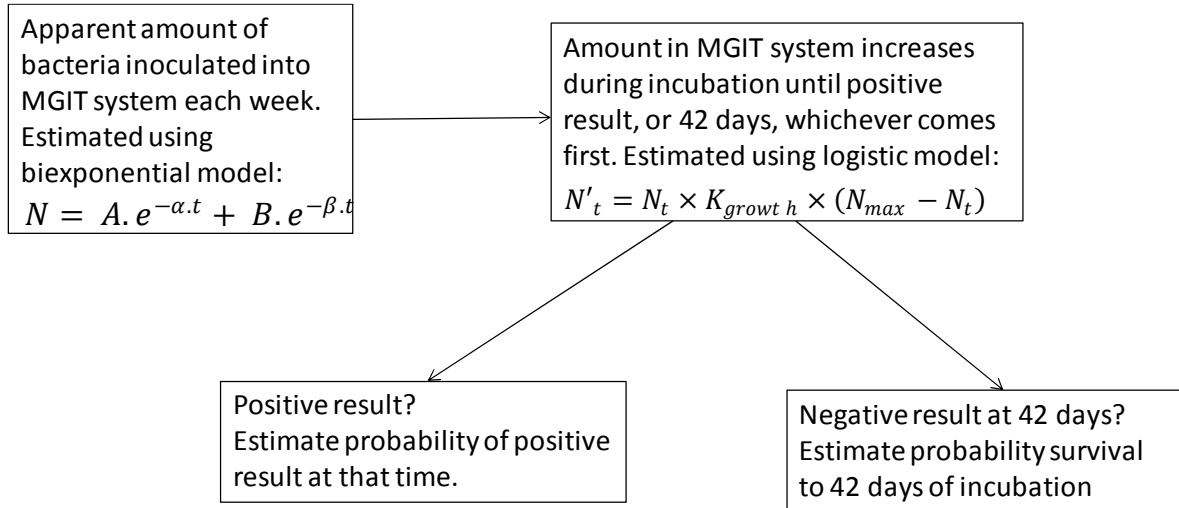


Figure 10.6: Schematic of final model and equations.

## 10.4 Discussion

This is the first semi-mechanistic model describing the progression of days to positivity of MGIT culture using a repeated time to event modeling approach and taking into account: 1) biexponential decline in bacillary load in patients, 2) differing reductions in the ability of the mycobacterial subpopulations to grow in the MGIT tube during treatment, 3) saturable growth in the MGIT system, and 4) the numerical nature of the MGIT data, i.e. the time-to-event type data. Although the time to positivity in MGIT data was highly variable, a general upward trend with time on treatment can be seen from the first figure, with the boxes plateauing late on treatment because of the censoring of the data at 42 days. The visual predictive check using Kaplan-Meier plots shows that the model can predict the observed data quite well.

To test potential use of the model, the effect of 2 covariates on some model parameters was investigated. The finding that patients with lung cavitation have higher amounts of bacteria that are killed slowly is in accord with previous studies that showed lower 2-month sputum

conversion rates (Burman *et al.* 2006; Visser *et al.* 2012) and a shorter time to detection (or days to positivity) (Perrin *et al.* 2010; Weiner *et al.* 2010) for patients with lung cavitation. The small number of patients with HIV infection may explain the failure to identify HIV infection as a significant covariate. However, other studies have also shown that HIV infection does not appear to have an effect on sputum conversion rates (Brindle *et al.* 1993; Senkoro *et al.* 2010; Brust *et al.* 2011).

Depending on the type of bacteria in culture, the model best describing their growth can differ (Zwietering *et al.* 1990), with the Gompertz model performing better than the logistic model in some cases (Gibson *et al.* 1987), or vice-versa (Annadurai *et al.* 2000). In the present case, the logistic model described the data better. It is probable that a positive MGIT culture result will have been recorded by the time the carrying capacity of the MGIT system is reached. Indeed, the model predicts that the carrying capacity could only be reached with the baseline sputum sample (data not shown); none of the subsequent samples having enough bacteria to reach the carrying capacity, even when cultured up to 42 days. None-the-less, the correct growth model must be used. The lag time that was estimated by the model is probably due to the bacteria having to recover from the harsh treatment of sodium hydroxide and the battery of other antibiotics used during the decontamination process of the sputum before the inoculum can start growing in the MGIT culture system. Another possible reason for a lag time would be post-antibiotic effects of the drugs administered to the patients during treatment. This presents a particularly important issue in that different drug classes may have differing post-antibiotic effects which could confound the association between time to positivity and the number of viable bacteria in the inoculum and negatively impact the ability of time to positivity to serve as a surrogate for the bacterial load when comparing the efficacy of different drug regimens. Care should be taken to address this possibility by testing the drug

regimens as covariates on the estimated lag times. A drug regimen with greater post-antibiotic effects would therefore have a longer lag time.

Although a biexponential model is presented here, a model with 3 exponents had an OFV that was 11 points lower than that of the biexponential model. However, due to the small difference in OFV upon addition of 2 parameters (intercept and slope) as well as wide confidence intervals from a bootstrap, the biexponential model was chosen since it was more stable. The marginally lower OFV for a tri-exponential model for bacillary decline in patients suggests the existence of at least a third bacterial population. Richer data than the current once weekly MGIT results may be able to properly define the third population. The difference between the OFV for the biexponential model and the monoexponential model was 29 points hence the biexponential model was significantly better and was deemed to describe data adequately.

A strength of the current model is that it can differentiate an increase in days to positivity due to killing of the bacteria in the patient, from an increase due to changes in the growth kinetics of the bacteria in MGIT culture. A decrease in the proliferative ability of the bacteria in the MGIT culture as time on treatment progresses was observed, suggesting a change in the fitness of the bacteria. A possible explanation for the decrease in proliferative ability with time is that as treatment progresses, only persister mycobacteria remain in the sputum and these may take a longer time to grow in the MGIT culture. Another possibility is drug pressure resulting in the selection for or acquisition of some mutations at a fitness cost (Cohen *et al.* 2003; Mariam *et al.* 2004; O'Sullivan *et al.* 2010; Rey-Jurado *et al.* 2011), although this does not occur indefinitely since evolution of compensatory mutations (Maisnier-Patin and Andersson 2004; Comas *et al.* 2011) is likely to occur. One other



possible explanation is development of an adaptive mechanism of change in expression of different pathways by the mycobacteria in response to the altered environment caused by drug pressure and other stresses. Population variability of 57% in the rate of change of the rate of bacterial growth rate in the MGIT culture was observed. A previous report found genetically similar mycobacteria obtained from different patients having different fitness (Gillespie *et al.* 2002), showing that host factors also play a role. The higher rate of declining fitness of the rapidly killed bacteria might be because of their faster rate of multiplication in the human host, such that they acquire mutations that impede fitness much faster than the bacteria that are already growing slowly since mutations can occur during DNA replication (Pray 2008).

Although this semi-mechanistic model predicts the data well, and is based on biologically plausible mechanisms, *in vitro* data to support the findings is lacking. Further work to determine the *in vitro* trends in growth rates and changes in the fitness of different subpopulations of *M. tuberculosis* during treatment is needed. To develop an even more mechanistic model experiments to identify a calibration curve to relate days to positivity from 0 - 42 with initial bacterial amounts, followed by identifying the exact (possibly non-linear) relationship between oxygen consumption and the hazard of a positive test result, would be ideal. It is also essential in this case to know the metabolic activity of the bacteria upon introduction to the culture. Identification of the exact threshold in oxygen tension and other factors that trigger a positive result would also enable more mechanistic models to be developed. An attempt to separate the bacterial growth compartment from the oxygen consumption compartment in the model was made. This resulted in a worse fit than the model we present. This however means that what is in the bacterial growth compartment with the logistic function is actually a combination of bacterial growth and oxygen consumption, and

that  $K_{\text{growth}}$  is a combination of the rate of bacterial growth and oxygen consumption. Trying to estimate between subject variability in the amount of bacteria could not be supported by the data and created model instability. Only one random effect could be estimated (as is typical for time to event type data) and this was on the change in viability of bacteria with time on treatment.

The tendency of increased variability in TTD with increased study length may be due to patients getting better resulting in more variable production of sputum in terms of both quality and quantity.

## **10.5 Conclusion**

In summary, a novel method for analysing the response of TB patients to treatment (and thus the pharmacodynamic properties of the combination drug regimen) using data comprised of days to positivity in MGIT cultures of serially collected sputum samples is presented. This model can be used to investigate covariates such as various drug regimens, drug exposure, strain of mycobacteria, patient/disease characteristics, or any other covariates on any of the model parameters in a manner similar to what has been shown with lung cavitation.

# **11.THE EFFECT OF ANTITUBERCULAR DRUG EXPOSURE ON DISEASE REGRESSION AND TREATMENT OUTCOME IN SOUTH AFRICAN PATIENTS WITH TUBERCULOSIS**

## **11.1 Introduction**

Literature contains a substantial amount of *in vitro* (Gumbo *et al.* 2007; Budha *et al.* 2009) and animal data (Jayaram *et al.* 2004; Nuermberger *et al.* 2004; Shandil *et al.* 2007) showing the importance of drug exposure on kill rates of *M. Tuberculosis*. However, most clinical study analyses (either full clinical trials or early bactericidal studies) are limited to comparison of different combination regimens (Sirgel *et al.* 2000; Burman *et al.* 2006; Dorman *et al.* 2009) or ranges of doses/dosing regimens (Anonymous 1976; Diacon *et al.* 2007; Zvada *et al.* 2012) rather than evaluation of the effect of individual drug exposures within regimens. Studies comparing different drug doses or different dosing regimens can be considered exposure-response studies since the dose given is a surrogate of expected exposure. Some studies of this nature have found a positive relationship between the dose of rifampicin and the rate of sputum conversion (response) in patients (Long *et al.* 1979). Dose-response curves (with response defined as the early bactericidal activity for the first two days) have also been characterised for a range of isoniazid and rifampicin dose in patients (Donald *et al.* 1997). On the other hand, some other dose-ranging studies have failed to show a relationship between doses and early bactericidal activity (Diacon *et al.* 2010), perhaps because the lowest dose of the drug in question (200 mg of PA-824 daily) was already resulting in the maximum kill rate.

Only a handful of studies have investigated the effect of individual drug exposures on patient treatment outcomes (Weiner *et al.* 2003; Weiner *et al.* 2005; Chideya *et al.* 2009). One study

found that patients with lower rifabutin (but not isoniazid) plasma concentrations were at greater risk of acquiring rifamycin resistance or relapsing after treatment (Weiner *et al.* 2005). Another study reported that low isoniazid concentrations were associated with treatment failure or relapse in a once weekly regimen with rifapentine (Weiner *et al.* 2003). A study conducted in Botswana found patients with a low pyrazinamide C<sub>max</sub> (which the authors defined as less than 35 mg/L) to have poorer treatment outcomes, with other drugs having no significant effect (Chideya *et al.* 2009).

What is evident from the inconsistencies in the study findings of the few clinical reports above, coupled with the fact that there have been no such studies conducted in the South African patient population, is that more studies to investigate exposure-response relationships are required, more so in the South African setting. Furthermore, none of the above studies were using sputum culture results from liquid media, hence results may differ due to differences in the methods of determination of the endpoints. We are only aware of one study that investigated differences between regimens based on time to positivity of liquid culture results (Diacon *et al.* 2010). Therefore, the aim of this work was to investigate the effect of the pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol on disease regression during treatment based on weekly MGIT liquid culture results in South African patients with TB. In addition, since the 2 month culture conversion endpoint will continue to be used until a better surrogate for sterilizing activity is found (Mitchison 1993; Burman 2003), another aim included investigation of drug exposure on the 2 month culture result.

## **11.2 Methods**

The group of study participants were being treated for ordinary TB with rifampicin, isoniazid, pyrazinamide and ethambutol as outlined in the methods section 4.1. A novel time to event

pharmacodynamic model using days to positivity in MGIT liquid culture was developed based on the 144 patients as described in the previous chapter (chapter 10). The model characterised the disease regression with time on treatment up to the 8<sup>th</sup> week, based on increasing days to positivity with the weekly sputum cultures. A total of seventy-three patients had both pharmacokinetic and pharmacodynamic data. The base model was rerun ‘as is’ on the subset of patients with pharmacokinetic data. The pharmacokinetic data was comprised of AUC<sub>0-24</sub> and C<sub>max</sub> for each of the four drugs, whilst the pharmacodynamic data was comprised of the weekly MGIT culture results. Using NONMEM, the AUC and C<sub>max</sub> for each drug were tested one at a time as covariates on the alpha and beta slopes of the biexponential disease regression model in patients (equation in section 10.3.1). Log-likelihood profiling implemented using PsN was used to obtain the 95% CI for the drug covariate of interest.

### 11.2.1 Classification And Regression Tree (CART) Analysis

CART analysis is a decision tree methodology first developed in the early 1980’s (Brieman *et al.* 1984). It is based on examining all possible ‘splitting variables’ and selects the one that results in binary groups that are most different from each other with respect to the dependent variable. It has been identified as an increasingly important research tool for identification of ‘at-risk’ populations in the health research field (Lemon *et al.* 2003). In this work, CART was used to identify variables predictive of the 2 month sputum culture result. The CART analysis was implemented in the CART<sup>®</sup> 6.0 Professional Extended package of the Salford Predictive Modeler Builder, (Salford Systems, San Diego, CA, USA) software. The variables of interest were the AUC’s and C<sub>max</sub>’s for each of rifampicin, pyrazinamide, isoniazid and ethambutol. Model evaluation included a cross validation testing phase which involved running an additional 10 trees each of which were tested on a different 10% of the data. The cross

validation results are then summarized automatically by the software and the output processed as part of the analysis. Another important model diagnostic is the Receiver Operating Characteristic (ROC) plot and the area under the ROC curve (Zweig and Campbell 1993). The area under the ROC curve is related to the Mann-Whitney-Wilcoxon test and it is the probability that a randomly chosen member of one class has a significantly smaller estimated probability of belonging to the other class than a randomly chosen member of the other class (Hand and Till 2001). It is thus a measure of separability between two estimated probability distributions (Hand and Till 2001). A random guess has an area of 0.5 (like a coin toss), whilst a perfect separation has a value of 1 (Zweig and Campbell 1993). A value of 0.7 or higher is deemed to be quite respectable (Steinberg and Golovnya 2007). Model diagnostics in this work included the area under the ROC curve for both the experimental dataset, and the test (cross validation) datasets. The ROC plot itself (only available for the experimental dataset) was also used as a diagnostic and it represents the fundamental ability of a test to distinguish between 2 possible outcomes hence is an index of pure accuracy (Zweig and Campbell 1993). Finally, confirmatory tests in the form of 2 x 2 contingency tables were carried out for the variables of interest from the final decision tree.

## **11.3 Results**

### **11.3.1 Time to event pharmacodynamic model implemented in NONMEM**

The final parameter estimates of the model run in this subgroup of patients were similar to those of the full dataset and are shown in the table below. The control stream of this model which is similar to the parent model is found in appendix 3.7.

Table 11.1: Population parameter estimates for model describing days to positivity in pharmacokinetic subset

Parameter	Estimated value
Baseline amount of rapidly killed bacteria	0.043
Rate of kill for rapidly killed bacteria ( $\text{week}^{-1}$ )	3.89
Baseline amount of slowly killed bacteria	0.00123
Fractional increase in baseline amount of slowly killed bacteria for presence of lung cavitation	0.745
Rate of kill for slowly killed bacteria ( $\text{week}^{-1}$ )	0.108
Bacterial lag time before growth in MGIT culture commences (days)	4.00
Baseline maximum growth rate of bacteria in MGIT culture ( $\text{day}^{-1}$ )	7.56
Rate of decrease of maximum growth rate in MGIT culture of rapidly killed bacteria ( $\text{week}^{-1}$ )	1.10
Rate of decrease of maximum growth rate in MGIT culture of slowly killed bacteria ( $\text{week}^{-1}$ )	0.308
Maximum carrying capacity of MGIT	0.18
Population variability in rate of decrease in maximum growth rate of bacteria in MGIT (%)	51

The rifampicin AUC was a significant covariate on the alpha slope, resulting in a (modest) 7 point decrease in the OFV, with a 1.8% (95% CI=0.85,2.8) change in the alpha slope for every 1 mg.h/L change in AUC from the median of 46 mg.h/L. The drug AUCs or Cmax for pyrazinamide, isoniazid and ethambutol were not significant on the model parameters. The VPC for the model with the rifampicin AUC covariate is shown below in figure 11.1. Like the parent model, it adequately captures the trends in the data towards increasing days to positivity with weeks on treatment.

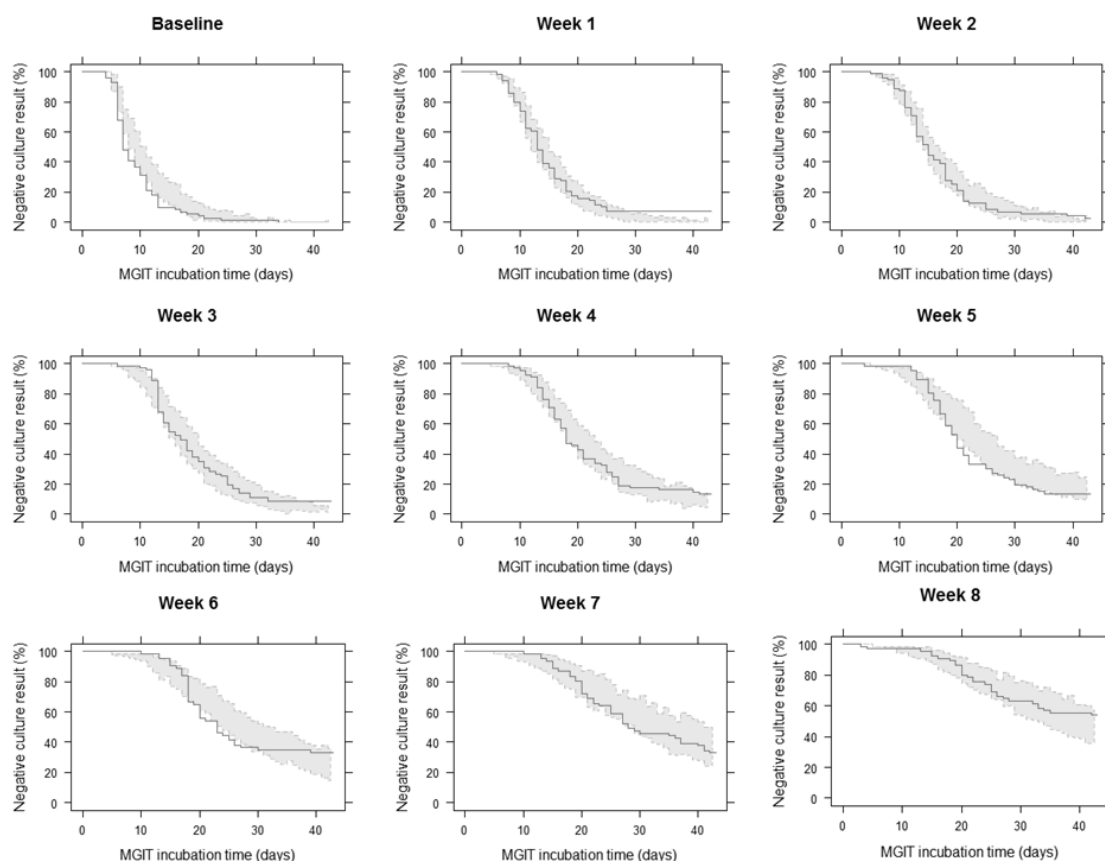


Figure 11.1: A visual predictive check from 100 simulations using the final model stratified into each week of treatment for the pharmacokinetic subset

### 11.3.2 CART Analysis of 2 month sputum culture results

CART analysis revealed that a rifampicin C<sub>max</sub> less than 8.55 mg/L was associated with higher 2 month culture positivity rates. The odds ratio for 2 month culture positivity in patients with a C<sub>max</sub> less than 8.55 mg/L was 5.6 (95% CI: 1.6-19.4;  $p < 0.01$ ). The second most significant predictor of the 2 month treatment outcome was ethambutol C<sub>max</sub>. For patients with a rifampicin C<sub>max</sub> less than or equal to 8.55 mg/L, an ethambutol C<sub>max</sub> greater than 2.34 mg/L was significantly associated with a positive sputum culture result, with an odds ratio of 4.0 (95% CI: 1.1-14;  $p < 0.05$ ). For patients with a rifampicin C<sub>max</sub> greater than 8.55 mg/L, an ethambutol C<sub>max</sub> greater than 4.23 mg/L was significantly associated with a positive sputum culture result, with an odds ratio of 4.0 (95% CI: 1.3-293;  $p < 0.05$ ). Thus high ethambutol exposure seems to be undesirable. The relative importance of ethambutol



compared to rifampicin was 0.36, meaning that ethambutol was about 3 times less influential compared to rifampicin. The final tree obtained is as shown in figure 11.2.

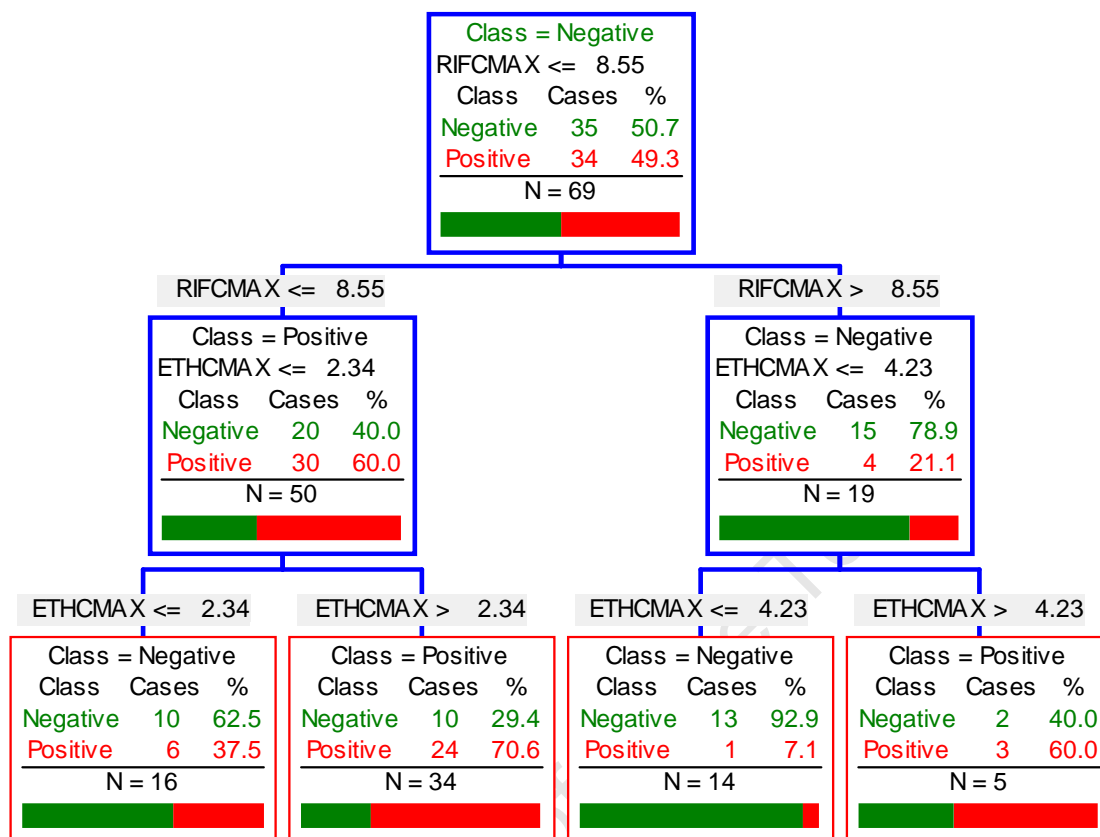


Figure 11.2: Final tree showing predictors of 2 month sputum culture status. RIFC MAX – rifampicin peak concentration, ETHC MAX – ethambutol peak concentration.

The area under the ROC curve for the actual data was 0.76, whilst for the test data (cross-validation) was 0.71. The ROC plots for the actual data are shown below.

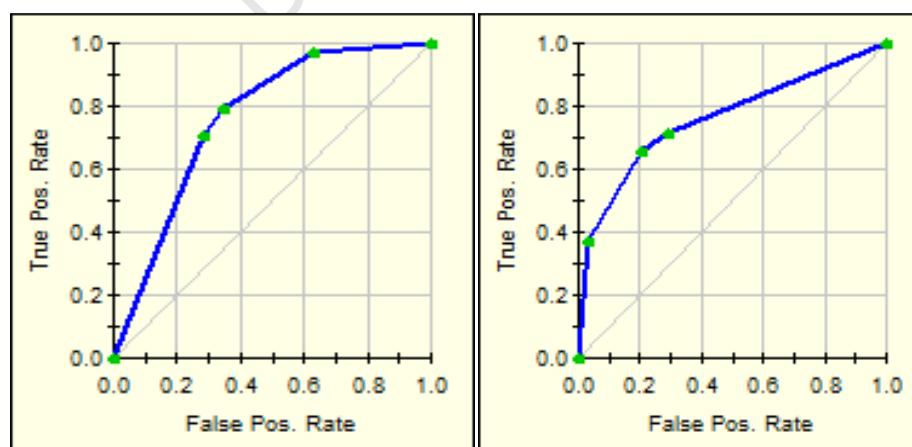


Figure 11.3: ROC plots for final CART model. The left one is for the positive class result whilst the one on the right is for the negative sputum results.

The table below shows the ability of the final model to correctly predict the 2 month culture status in patients.

Table 11.2: Prediction success percentages for final CART model on the actual dataset and the cross validation dataset

Class	% correct on actual dataset	% correct on test dataset
Negative	66	66
Positive	79	76
Average	72	71

The model's ability to predict the 2 month culture status appears to be slightly better for individuals with a positive result compared to those with a negative result. The average percentage misclassification is 28% (calculated from 100-72) for the actual dataset, and 29 for the test dataset. Generally, the CART model diagnostics show that the model can predict an individual's 2 month culture status with good accuracy.

## 11.4 Discussion

This is one of the few studies that have investigated the effect of individual measures of drug exposure on disease regression and treatment outcomes in patients. The finding that rifampicin AUC significantly influenced the alpha slope is consistent with other reports from EBA studies that found increased rifampicin exposure to result in lower bacterial counts after 2 days of treatment (Diacon *et al.* 2007). It is however unlikely that this affects final outcome since most of the rapidly killed population is gone after the first few days of treatment. The reported alpha slope of 3.89 week<sup>-1</sup> translates to a time to kill half of the 'alpha population' of 1.25 days, which means little from a sterilizing perspective. Failure to find statistically

significant relationships apart from the rifampicin AUC may be due to the limited sample size and narrow dosing ranges per unit bodyweight since weight-based dosing was used.

The CART analysis result of a rifampicin C<sub>max</sub> cut-off value of 8.55 mg/L supports a previously recommended minimum of 8 mg/L in a therapeutic drug monitoring laboratory (Peloquin 2002). This work now provides strong clinical evidence in favour of that report. This also suggests that the *SLCO1B1* rs4149032 polymorphism may be partly responsible for the lower cure rates observed in African populations compared to non-Africans (Burman *et al.* 2006; Dorman *et al.* 2009) since the SNP reduces rifampicin bioavailability. Chapter 5 discusses that the majority of our study population had peak concentrations below 8 mg/L and recommends higher doses in this African population. This work further enhances that argument. Rifampicin AUC was not found to be a significant predictor of the 2 month culture result, unlike the C<sub>max</sub>. Since the two variables were reasonably correlated in this study ( $R^2 = 0.59$ ), another CART analysis was run ignoring the rifampicin C<sub>max</sub>. However, the rifampicin AUC still did not appear to be of any statistical significance and could not replace the C<sub>max</sub>. This means that C<sub>max</sub> was not identified as a surrogate for the AUC, but that it is actually the C<sub>max</sub> that is the primary driver of the 2 month outcome. Apart from the EBA studies which have shown importance of rifampicin AUC, the AUC has also been reported to be a better predictor of long term outcome (e.g. relapse or death in patients followed for up to 2 years), whilst the C<sub>max</sub> is more important for shorter term outcome like the 2 month sputum culture status (Pasipanodya *et al.* 2012). The C<sub>max</sub> of rifampicin is associated with the post-antibiotic effect and whether or development of resistance will occur (Gumbo *et al.* 2007), both of which are important in the early months of treatment.

Whilst the C<sub>max</sub> cut-off for this study compares well with the value of 8 mg/L, it is slightly higher than the value of 6.6 mg/L which has been reported in the only other CART analysis of TB drugs we are aware of (Pasipanodya *et al.* 2012). Although the 2 values are comparable, a possible explanation for the difference may be that our metric for 2 month culture status is based on MGIT liquid culture, whilst for Pasipanodya *et al.* it was solid culture.

Pyrazinamide peak concentrations have been recently reported to be the best predictor of 2 month culture status, with a cut-off value of 58.3 mg/L (Pasipanodya *et al.* 2012). In our study, only 2 patients had a C<sub>max</sub> greater than 58.3 mg/L, thus almost all patients were already below the recommended minimum C<sub>max</sub>. Since this dichotomizing metric was practically absent in our cohort, this may explain why pyrazinamide exposure was not of significance, apart from a potential effect of different culture media once again.

An interesting (paradoxical) finding is that higher ethambutol exposures were associated with a higher chance of having a positive 2 month sputum culture result. This suggests a possible antagonistic effect of ethambutol which has been previously reported with rifampicin *in vitro* (Dickinson *et al.* 1977) as well as *in vivo* when combined with other drugs including rifampicin in multi-drug regimens (Jindani *et al.* 2003). Conversely, synergism between rifampicin and ethambutol on *Mycobacterium avium* complex has been reported (Heifets *et al.* 1988) thus our findings require further investigation.

## 11.5 Conclusion

The time to event pharmacodynamic model based on days to positivity in liquid culture developed in chapter 10 was successfully implemented to investigate the effect of the

pharmacokinetics of the drugs on the kill rates of the mycobacteria in patients, although only rifampicin had an effect on the alpha slope which is unlikely to be of clinical significance. Based on CART analysis, a rifampicin C<sub>max</sub> of 8.55 mg/L is proposed as the minimum target that patients should achieve during treatment since higher values than this cut-off are associated with higher 2 month sputum culture conversion rates. According to the previous simulations (chapter 5), this translates to necessitating a roughly 30% increase in the daily dose (150 mg) for the majority of South African patients. The potential antagonistic effects of ethambutol and failure to find significant effects of the other drugs require further investigation in larger studies.

## **12. CLINICAL TRIAL SIMULATION TO OPTIMIZE STUDY DESIGN FOR EVALUATING THE EFFICACY OF ANTITUBERCULAR DRUGS**

### **12.1 Introduction**

Clinical trials have become the standard way to inform decisions (often at a regulatory agency level or at the level of a pharmaceutical company) about what the best treatment for a patient would be amongst multiple options (Sheiner and Rubin 1995). Over the past two decades, as computational power has increased and mathematical models predicting pharmacokinetics and pharmacodynamics have improved, the simulation of clinical trial (Holford *et al.* 2000) outcomes has gained popularity because it allows prospective testing of different possible clinical trial designs as various simulation scenarios. Using simulation, this optimization of clinical trial design can be done before actually carrying out the study (Lockwood *et al.* 2006) thereby increasing the chances of a clinical trial successfully addressing the research question (Bonate 2000).

Most clinical trials investigating the response of TB patients to drug therapy have been conducted as parallel studies (Bock *et al.* 2002; Burman *et al.* 2006; Diacon *et al.* 2009). Crossover designs are uncommon because: 1) a washout period is unethical because discontinuation of their medication would be detrimental to patients, and 2) there are changes in the bacterial population during treatment with one drug regimen, which would confound the effect of a subsequent regimen. Still, crossover studies are commonly used to assess drug-drug interactions (McIlleron *et al.* 2007; Zvada *et al.* 2010), and bioequivalence (Pillai *et al.* 1999). They have demonstrated greater statistical power relative to parallel designs for studies assessing efficacy of interventions for Alzheimer's (Lockwood *et al.* 2006) and Parkinson's disease (Putt and Ravina 2002) when the magnitude of inter-individual

variability was greater than intra-individual variability for the clinical response. Whether a parallel or crossover design, any antitubercular drug trial can fail. Typically, the reasons for trials failing can be related back to one or more of the following: 1) the study duration may be too short thereby missing a significant drug effect (Jindani *et al.* 2003), 2) the sample collection times e.g. sputum collection, may be suboptimal producing lower than desired pharmacodynamic effects, thus reducing power (Davies *et al.* 2006), 3) use of a suboptimal dose, thus reducing effect size 4) insufficient enrolment, resulting in a fewer participants than required an based on statistical power calculations (Bonate 2000), or 5) lower bactericidal activity than was anticipated, resulting in reduced effect size.

Modeling and simulation approaches can inform trial designs to reduce the typical reasons for failure. For example, a NLME modeling analysis of serial sputum colony counts determined during anti-tubercular treatment proved to be a significant improvement and more powerful analysis method than conventional methods when distinguishing mycobacterial kill rates in combination regimens (Davies *et al.* 2006). For these complex types of modeling analyses, sample size and study power calculation methods are not commonly available and are relatively complex (Kang *et al.* 2004) compared to using conventional statistical tests (e.g. t-tests). There is therefore a need to determine the optimal study design and sample size when a modeling approach to data analysis will be employed.

It is known that current antitubercular drugs within the multi-drug regimen have distinct activities against the different mycobacterial subpopulations in patients resulting in diverse kill rates (Jindani *et al.* 2003). It is generally accepted that to achieve the goals of shortening treatment and improving cure rates, drugs with increased sterilizing activity (i.e. with increased activity against persisters) are required (Ibrahim *et al.* 2009). With the increasing

numbers of antitubercular drugs entering clinical development, and with the increasing number of calls for optimization (increases) of the doses for the drugs currently in use, there is a need to ensure that clinical trials are designed in an efficient manner to ensure reliability of study findings and validity of results and the conclusions drawn.

The objective of the simulation experiment was to determine an optimal trial design to compare the sterilizing effect of two antitubercular treatment regimens on viable mycobacteria in serially collected sputum samples. Design properties to be optimized included trial duration, number of study participants and trial type, i.e., parallel or crossover. The optimization metric was based on sufficient power to correctly reject the null hypothesis i.e. there is no difference between the test and the control regimens. This was to be determined using a NLME modeling approach for the data analysis taking into account a realistic clinical setting in which protocol deviations such as missing samples occur, there are issues such as the limit of quantification of assays to consider, and high variability in the measurements of treatment response.

## **12.2 Methods**

The purpose of the simulation study was to determine the ability of a clinical study to detect a statistically significant increase in the  $\beta$  slope (sterilizing phase) for the (hypothetical) test regimen compared to the standard regimen.

### **12.2.1 Simulation Model and Parameter Settings**

The simulation model was based on a phase II study investigating the sterilizing activities of various fluoroquinolones (moxifloxacin and gatifloxacin) compared to ethambutol in patients with pulmonary TB also receiving rifampicin, pyrazinamide and isoniazid in the 8-week



intensive phase of treatment, as reported by Rustomjee *et al* (Rustomjee *et al.* 2008). Important aspects incorporated in our model include a biexponential model describing the decline in CFU counts/mL in sputum processed from patients during treatment was implemented in the form of the equation below:

$$\text{Log}_{10} \text{ CFU/mL} = \text{Log}_{10}(10^A \times e^{-\alpha.t} + 10^B \times e^{-\beta.t}) \quad \text{Equation (1)}$$

where A is the baseline concentration of bacteria that are killed rapidly (6.99),  $\alpha$  is the rate of kill of these bacteria ( $1.2 \text{ day}^{-1}$ ), B is the baseline concentration of bacteria that are killed slowly (5.79),  $\beta$  is the rate of kill of these bacteria ( $0.309 \text{ day}^{-1}$  for the standard drug regimen, and a value of  $0.389 \text{ day}^{-1}$  for the test regimen i.e. 26% increase in the kill rate), and t is the time on treatment in days.

Log transformation was necessary to address numerical instability caused by the wide range in orders of magnitude of the data. The above typical input values are derived from those reported by Rustomjee *et al* (Rustomjee *et al.* 2008). The parameterization is however slightly different in that the  $\alpha$  and  $\beta$  values are not exponentiated thus the kill rates can take on positive values (vast majority) but may also have negative values meaning that it is possible for the odd patient to get worse on treatment. This is more realistic than assuming that all patients will get better on treatment. Consequently, it was necessary to adjust the kill rates to match those from Rustomjee *et al* after their exponentiation. As the authors did not report values for the population variability in the parameters (although they reported that it was 'high'). Estimates of the variability for the various model parameters were obtained from other published studies (Desjardin *et al.* 1999; Joloba *et al.* 2000; Brindle *et al.* 2001; Davies *et al.* 2006; Diacon *et al.* 2007). Variability in the baseline concentration of bacteria was 12% (Joloba *et al.* 2000), which was considered similar to the 9% (Desjardin *et al.* 1999) and 6% (Diacon *et al.* 2007) reported in other studies. The highest variability was chosen in order to

be more conservative. This variability was placed only on the bacteria that are killed rapidly (the 'A' population) because the estimation model had identifiability problems in separating this variability from variability in the much smaller 'B' population. Since the published study (Rustomjee *et al.* 2008) did not estimate variability in  $\alpha$  due to estimation difficulties, this was also omitted from the model. For the simulations, variability in the  $\beta$  slope was set to 62% (Brindle *et al.* 2001; Davies *et al.* 2006).

To account for parameter uncertainty and measurement error in the measurement of  $\log_{10}$  CFU/mL counting, a 22% coefficient of variation was included in the model (van Zyl-Smit *et al.* 2011). The LLOQ for counting was set at 1.0  $\log_{10}$  CFU/mL (Rustomjee *et al.* 2008) and was similar to 0.699  $\log_{10}$  CFU/mL reported elsewhere (Yajko *et al.* 1995). A 0.059 probability of missing CFU counting results due to lost or contaminated samples (Rustomjee *et al.* 2008) was included as part of efforts to mimic the realistic clinical setting. The sputum sampling scheme was taken from Davies *et al.* (Davies *et al.* 2006), who determined the optimal sampling times for serial sputum colony counts over a wide range of kill rates using a NLME analysis approach. The sampling times were at baseline, after 2 days, then weekly for 8 weeks (days 0, 2, 7, 14, 21, 28, 35, 42, 49, 56) and these are the same time points that were used in the actual clinical study (Rustomjee *et al.* 2008) conducted by Rustomjee *et al.*

### 12.2.2 Clinical Trial Simulation

The clinical trial simulation was implemented using Pharsight® Trial Simulator 2.2.1 (Pharsight Corporation, Mountain View, California, USA). Two hundred and fifty replicates for each simulation scenario were carried out. The simulation output was comprised of  $\log_{10}$  CFU/mL counts for each sampling time point (except for missed samples) for each study participant, as well as the study arm for each patient.

### 12.2.3 Simulation scenarios

#### 12.2.3.1 Parallel study design

The base clinical trial simulation included 100 patients randomized to one of 2 study arms (control arm and test arm) in a 1:1 ratio i.e. a parallel design study with 50 patients in each arm. The number of patients in the study was then increased until a study power greater than 0.80 was observed. This was a power to detect a difference (increase) in the  $\beta$  kill rate between the test compared to the control regimen. The duration of this trial was 56 days (8 weeks) and sample data was simulated according to the optimal sampling scheme described above. To determine the effect of the duration of the study on the study power, shorter trial durations (42, 35, 28 days) were tested (though still based on the sampling scheme described above).

#### 12.2.3.2 Crossover study design assuming no carryover effects

Since a modeling approach for data analysis can address some of the pitfalls of a crossover study, various randomized 2-period 2-sequence crossover study designs were investigated (as described in the next section). Various study durations and the time at which switching of regimens took place (not only the mid-point of the study) were also tested. The crossover design was implemented using Equation (1) up to the time of switching regimens and thereafter using the equation below:

$$\text{Log}_{10} \text{ CFU/mL} = \text{Log}_{10}(10^{A2} \times e^{-\alpha \cdot t2} + 10^{B2} \times e^{-\beta \cdot t2}) \quad \text{Equation (2)}$$

where A2 and B2 are the corresponding concentrations of rapidly killed and slowly killed bacteria at the time of switching, t2 is the study time less the time at which the switching took

place, and  $\beta_2$  is the new period-dependent sterilizing kill rate, i.e., dependent upon the patient randomized sequence.

### 12.2.3.3 Crossover study design incorporating pharmacokinetic carryover effects

The base crossover model assumed no carryover effect from one drug regimen to the next. This is probably true for drugs with a short half life and a short post-antibiotic effect. However, many of the new drugs have long half lives e.g. PA824 at 16-20 hours (Ginsberg *et al.* 2009), delamanid at 38 hours (Gler *et al.* 2012) and TMC207 (bedaquiline), which has an extremely long half life (Andries *et al.* 2005). To investigate the effect of changing regimens from a more effective test drug with a long half life to the less effective regimen, a gradual decline of the kill rate ( $\beta$ ) from the more effective regimen ( $\beta_{\text{test}}$ ), tapering off to the lower kill rate ( $\beta_{\text{control}}$ ) was implemented using the equation below:

$$\beta_2 = \beta_{\text{control}} + (\beta_{\text{test}} - \beta_{\text{control}}) \times e^{-\frac{0.693}{t_{\text{half}}}.t} \quad \text{Equation (3)}$$

where  $t_{\text{half}}$  is the half life of the carryover effect of the test regimen and  $t$  is the time after switching treatment to the control regimen.

Assuming it will take 5 half lives for the carryover effect to wear off, a carryover effect lasting 5 days ( $t_{\text{half}}=1$ ) and another lasting 10 days ( $t_{\text{half}}=2$ ) were investigated. Post-antibiotic effects of up to 5 days have been reported *in vitro* (Chan *et al.* 2001). The above scenario meant that in the estimation, drug regimen as a covariate on  $\beta$  had to be tested (as before) but in addition to estimating the half-life of the carryover effect (hence 2 extra parameters compared to the base model).

#### 12.2.4 Nonlinear mixed effect modeling analysis

Since the intention of the exercise was to determine the difference in the  $\beta$  slope between the test and the control regimens, a regression process was necessary to determine the slopes for the 2 regimens. The simulation output from Pharsight® Trial Simulator was post-processed using R version 2.15.1 (R\_Development\_Core\_Team 2008) before being transferred to Phoenix NLME® 1.3 (Pharsight Corporation, Mountain View, California, USA) for nonlinear mixed effects modeling to estimate all of the model parameters.

The base estimation structural model in each case was that used for each simulation scenario as described above whilst the covariate model included study arm (test or control regimen) as a covariate on the  $\beta$  slope. The Laplacian estimation method was used. Data below the LLOQ were included using a cumulative distribution function for the normally distributed error to calculate the likelihood that they were below 1.0 log<sub>10</sub>CFU/mL. This method has previously been shown to be the best method of handling such data compared with imputations or discarding the data (Ahn *et al.* 2008). A log-likelihood ratio test was then performed for each clinical trial replicate to determine whether inclusion of drug regimen as a covariate was of statistical significance. A reduction in the objective function value of at least 3.84 points for each additional estimated parameter in the covariate model compared to the base model was considered statistically significant (P=0.05). The study power for each simulation scenario was calculated as the proportion of replicates detecting a statistically significant drug effect that had been inherently included in the simulations. For the best parallel and best crossover studies, the probability of a type 1 error occurring was subsequently determined. This was undertaken by simulating a clinical trial with the  $\beta$  slope for the test regimen being equal to that for the control regimen, then determining the proportion of modeling results which found a statistically significant difference between the 2 study arms.

## 12.3 Results

Based on the biexponential model, the figure below illustrates the typical concentration time profiles for the parallel study as well as for the crossover study with treatment switching taking place at 28 days.

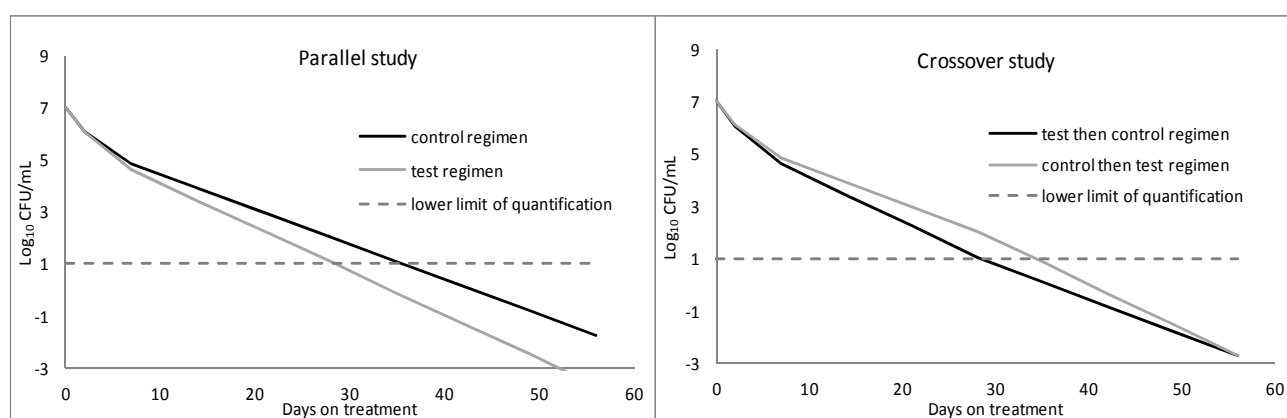


Figure 12.1: Simulated typical bacterial concentration-time profiles for the test (moxifloxacin, rifampicin, isoniazid, pyrazinamide) regimen and control (ethambutol, rifampicin, isoniazid, pyrazinamide) regimens depending upon study design. The time at which treatment was switched for the crossover study in this case was 28 days.

For all the simulated studies, there was good agreement between the parameter estimates used for the simulation in Pharsight® Trial Simulator and those found from the estimation process in Phoenix NLME® including the estimates of between subject variability, hence the simulation-estimation process was deemed reliable. However, the estimate for the test regimen effect on the  $\beta$  slope in the crossover studies tended to have a (small) average bias of -5% and the  $\beta$  slope of the control regimen had a bias of +6%. All other estimates were practically identical to those used for the simulations. The average bias for the test regimen effect on the  $\beta$  slope in the parallel study was less than  $\pm 1\%$ . The table 12.1 shows the increasing percentage of observations that were below the lower limit of quantification of 1.0

$\log_{10}$  CFL/mL as time on treatment increased for the base clinical trials (100 study participants and study duration of 56 days).

Table 12.1: Percentage of observations below the LLOQ of 1  $\log_{10}$  CFU/mL for each sampling time point in the base parallel and crossover studies.

Sampling time (day)	Parallel study	Crossover study
0	0	0
2	0	0
7	0	0
14	9.5	9.7
21	25	26
28	42	42
35	56	56
42	67	67
49	76	75
56	82	82

The overall percentage of data below the LLOQ for both study designs was 36% after correcting for the 6% missing data. The table shows a steady increase in the amount of data that was below the LLOQ with increasing time on treatment, but with no difference with respect to the study design.

### 12.3.1 Simulation scenarios

#### 12.3.1.1 Parallel study design

With a parallel study design and sputum samples taken up to 56 days, 100 and 300 patients yield a study power of 42% and 89% respectively. The table below shows the study power for various study lengths for a parallel study designs using 200 patients.

Table 12.2: Study power for various study durations for parallel study design using 200 patients

Number of patients	56 days study duration	42 days study duration	35 days study duration	28 days study duration
200	0.69	0.74	0.75	0.73

The ideal parallel study required about 250 patients followed up for 35 days, which resulted in a study power of 85% and a type 1 error rate of 1%. Interestingly, a study length of 56 days had a lower statistical power than that lasting only 42 or 35 days.

#### 12.3.1.2 Crossover study design assuming no carryover effects

The base crossover study had 100 patients with a 56 day study duration and treatments switched at 28 days in one study, and 21 days in another study. Both had a study power of 1.0. The number of patients was subsequently reduced until study powers around 0.80 were reached before testing other scenarios. Even 40 patients for the crossover study (with switching at either 21 or 28 days) still gave a study power close to 1.0, until a number of 20 patients was reached which was then used for the simulation scenarios shown in the table 12.3 which shows the study power for various scenarios of a crossover study for a drug with no carryover effect.

Table 12.3: Study power for different study duration and different switching times for crossover study design with no carryover effect

Number of patients	42 days study duration	35 days study duration	28 days study duration
20 (crossover at 21 days)	0.82	0.82	
20 (crossover at 14 days)	0.89	0.90	0.84



Twenty patients followed up for the full study duration (56 days) gave a study power of 0.80 when the crossover took place at 28 days, whilst the power was 0.41 for a study lasting 35 days (still switching treatments after 28 days). The phenomenon of lower statistical power with increasing study length beyond 42 days which was observed with the parallel study was repeated in the crossover studies although this was dependent upon when the crossover took place. The best crossover design had 35-day study duration with treatments being switched after 14 days. This design gave a power of 0.90 with 20 patients and a type 1 error rate of 0.04.

#### 12.3.1.1 Crossover study design incorporating pharmacokinetic or post-antibiotic carryover effects

The best crossover design above was subsequently used to investigate the carryover effects described in the methods section above. For both the carryover lasting 5 days and that lasting 10 days, the study power decreased to 73%, hence more patients would be necessary to restore the desired study power. Increasing the sample size by 50% to 30 patients then restored study power to 88%.

## **12.4 Discussion**

The simulations performed demonstrate that it is possible to improve the design and analysis of clinical trials in TB to ensure validity of findings and to enhance the efficiency with which new regimens can be developed. A simple example why this should be the case is based on a study that failed to show a difference in the substitution of moxifloxacin for ethambutol based on the ‘snapshot’ of 2 month sputum culture status in 277 patients (Burman *et al.* 2006). Another study based on the same analysis approach in 328 patients also failed to find a significant effect of moxifloxacin (Dorman *et al.* 2009). On the other hand, a very similar

study with only 209 patients found a significant effect of moxifloxacin and the method of data analysis compared the rates of mycobacterial kill using a modeling approach (Rustomjee *et al.* 2008) for serially collected sputum colony counting results. This shows that it is time more thought was put into the design of TB clinical trials.

Using clinical trial simulation, an investigation of various study designs to provide more information for research teams planning future studies was carried out. It must be emphasized that these study results are based on a design of substituting one drug with another (replacing ethambutol with moxifloxacin in the test regimen) and comparing that to the control regimen (standard treatment) which is the current gold standard approach. The modeling approach to the data analysis makes the crossover study design more conceivable bearing in mind possible effects the first drug regimen may have on the bacteria that may affect their sensitivity to the regimen administered after switching has taken place. A crossover design comparing 2 completely different regimens may be compromised by potentially irreversible bacterial changes caused by one or the other regimen (or both) and may have findings that are difficult to interpret. In this case some *in vitro* data would be needed to justify modeling and simulation assumptions.

For the simulation study, the overall percentage of data below the limit of quantification was strikingly similar to the 34% that was observed in the actual clinical study (Rustomjee *et al.* 2008) upon which the simulations were based. This gives confidence in the simulations which used inter-patient variability estimates from other literature sources, and also supports the authenticity of the published trial. The agreement between the simulation parameter estimates used in one software program and the estimated parameters in another program also helps to validate the approach. The slightly reduced effect size observed for the test regimen

on the  $\beta$  slope in the crossover design may be due to half the number of sampling points are available for use in the regression process for a given study period which may reduce the ability of the model to differentiate between the differences in the slopes between the 2 periods.

It is generally thought that the worst possible effect of following up patients longer than necessary is increased study costs and that it may perhaps also be unethical. This simulation study however shows that unnecessarily increasing study duration may actually compromise study power as well, although the decline in power was marginal. At first sight this may be surprising especially without considering the increasing percentage of data below the LLOQ with time on treatment (which was not discarded in the analysis). A possible explanation is that once the data points below this limit begin to occur in succession, it is then difficult to differentiate the slope between one regimen from that of another since the data points in both cases have no residuals and may have an equal likelihood of being less than the LLOQ. Increased study duration also has a potential problem of increasing patient dropout due to various reasons although the simulations did not take this into account.

Further simulations were carried out to take into account the possibility of a drug regimen having some carryover effect after treatments have been switched. The results show that this is accompanied by a decrease in study power and a significant increase in the number of study participants by up to 50% would be required to maintain the original targeted power. It is interesting to note that the duration of the carryover effect had no influence on the study power, which was the same for an effect lasting about 5 days compared to an effect lasting about 10 days. Of much greater importance is correctly taking this into account in the modeling process. Although a larger sample size was needed for the carryover study, 30

patients is nowhere near the 250 patients needed to attain similar study power for the parallel study. Some carryover effects (particularly pharmacokinetic carryover) may wear off as implemented in the modeling analysis. On the other hand, it is possible that some regimens may have irreversible pharmacodynamic effects and this requires thoughtful consideration in the modeling and simulation process.

The simulations did not include any within subject variability in the model parameters since this has not been described in literature, coupled with implementation difficulties in the software. However, it is possible that an observed variability as high as 62% in the  $\beta$  slope may include some within subject variability, hence the sample size estimates for the crossover study should be interpreted with caution although this should not be a problem for the parallel study.

The low type 1 error rates observed show that the chances of falsely identifying the presence of a difference in the  $\beta$  slopes between the test and the control regimen are very low.

The ideal numbers of patients and study duration for parallel and crossover studies based upon previously reported mycobacterial kill rates in the current standard regimens and an effect size of about 25% have been identified. However, as drug regimens improve and become more potent, it is possible that the current optimal design reported may change. Thus some prior knowledge the expected potency and kill rates of a regimen would help inform research teams on altering their study design accordingly. An unfortunate reality is that extrapolation of observed *in vitro* kill rates to obtain expected kill rates *in vivo* may be quite challenging. It is probable that more effective regimens would require shorter study durations

since increasing amounts of data below the limit of quantification leads to decreased statistical power.

#### **12.4.1 Limitations**

In general, concentration-effect relationships have not been identified *in vivo* for anti-tubercular drugs, hence it is difficult to imagine reasonable values to include in the simulations in order to enable implementation of simulations using full pharmacokinetic-pharmacodynamic models. Another (less likely) scenario which not addressed is that the weaker control regimen may cause the bacteria to be less susceptible (e.g. through induction of efflux pumps) to the test regimen which will be introduced at a later stage. This may then render the subsequent regimen less effective during the second period than if the weaker regimen had not been administered in the first place. Should such a scenario occur, it can however be addressed through a modeling approach by estimating a study sequence dependent change in  $\beta$ . While we have managed to account for pharmacokinetic carryover in crossover studies, pharmacodynamic carryover may be more complex and needs further investigation and *in vitro* data to assist the modeling on a case by case basis.

#### **12.5 Conclusion**

An approach that optimizes the design of clinical trials investigating new drug regimens based on a NLME modeling data analysis approach is presented. About 250 patients are needed for a parallel study using optimally designed sampling points and a modeling analysis approach to detect a difference of about 25% in the terminal slope. These kind of studies could replace Phase II studies to select best regimens to take forward into more robust phase

III studies. Crossover studies may be considered as a means to improve study power and reduce sample size (hence clinical study financial and ethical costs), acknowledging that such studies may only be useful when a modeling approach is used for the data analysis and that they need further evaluation against parallel studies before they can substitute parallel studies. Clinical studies should not be unduly long otherwise this may compromise study power. The clinical trial simulation approach can be used to investigate other possible simulation scenarios such as different effect sizes or different between subject variability in model parameters, or other study designs such as factorial designs.

University of Cape Town

### 13. OVERALL DISCUSSION AND CONCLUSIONS

TB continues to be a large global health problem, with a high proportion of infections and deaths occurring in the African population of which South Africa is considered a highly burdened country. In fact, South Africa has by far the highest incidence of TB infection in the world, with an incidence of up to 1180 infections per 100 000 people in 2011 (WHO 2012). It is therefore of utmost importance to conduct research to improve our understanding of the pharmacology of the drugs currently in use as part of efforts towards optimization of treatment in this high burden environment. It is imperative to use the available drugs optimally especially considering the fact that low drug exposure can lead to development of resistance (Gumbo *et al.* 2007). Even though new drugs may be on their way, it is important to develop and implement methodologies (e.g. analysis of TTD data) that will allow critical evaluation and dose optimization of the new regimens in our clinical setting to ensure that these drugs are used optimally. This would help reduce the emergence of resistance since drug resistant TB is more difficult to treat and requires 18-24 months of drug administration. Even for MDR-TB itself, little is known about the adequacy of the doses used in our population, particularly for those drugs whose efficacy may not be limited by toxicity with the current dose ranges (fluoroquinolones). Apart from fluoroquinolones and pyrazinamide, doses of the other second line antitubercular drugs are limited by severe toxicity hence PK-PD work to suggest dose increments may be futile e.g. for aminoglycosides. The important role of fluoroquinolones, their relatively wider therapeutic margins and previously published *in vitro* PK-PD work to identify target AUC/MIC ratios make them good candidate drugs for investigation of dose optimization in MDR-TB. At the same time, there are very few studies that link the AUC with the MIC in the same patients.

This thesis adds significantly to the body of knowledge in terms of the pharmacokinetics and pharmacodynamics of antitubercular drugs based on data collected from patients in routine clinical settings in South Africa. The drug regimens and combinations given to patients were the standard regimens used in many parts of the world, hence the findings may be generalizable beyond South Africa. Most of the patients were HIV negative, did not have unique/special characteristics, and were not receiving drugs expected to alter the generalizability of the findings. The work involved describing the pharmacokinetics of the first-line drugs in South Africans to augment the limited previous reports in this patient population and identify factors influencing interpatient variability. Another aim was to investigate the effect of individual drug exposure on disease regression and patient outcomes after 2 months of treatment as determined using the pharmacokinetic models built as part of the previous aim. Before this could be done, it was necessary to develop a model describing the pharmacodynamics of the combined drug regimen based on the relatively sensitive measurement of days to positivity in MGIT culture. The time to event pharmacodynamic model could then be used to investigate covariates such as drug exposure.

Further work in patients with MDR-TB involved the previously unstudied area of ofloxacin pharmacokinetics in South African patients with the aim of assessing the adequacy of ofloxacin exposure in relation to *M. tuberculosis* susceptibility profiles. Numerous studies have called for investigation of the efficacy of higher doses of various antitubercular drugs (Gumbo *et al.* 2004; Diacon *et al.* 2007; Gumbo *et al.* 2009), especially since some of the proposed higher doses appear to be safe (Ruslami *et al.* 2007; Pasipanodya and Gumbo 2010). The findings from the rifampicin and ofloxacin models in the current study add to these calls for re-evaluation of TB drug doses and dose optimisation. Clinical trials are the standard way of evaluating the efficacy of one regimen compared with another. However,



there is a need to ensure that these trials investigating the recommended higher doses, or those simply investigating a new drug, are conducted as efficiently as possible. Thus the final aim was to determine the optimal study design of an antitubercular drug trial using clinical trial simulation. More informative clinical trials and more efficient comparison of candidate drugs regimens would ultimately lead to more rapid progress of these new drugs to the clinic. The key findings, limitations, recommendations and scope for further work in the above areas are discussed in relation to the project objectives, below.

### **13.1 What Are The Pharmacogenetic Determinants of Rifampicin Pharmacokinetics and What Does This Mean for South Africans?**

A novel finding of this work was that polymorphism of an influential drug transporter in rifampicin disposition resulted in a decreased bioavailability of the drug. The *SLCO1B1 rs4149032* polymorphism existed at a high frequency of 0.70 in our South African patient cohort. Fifty-two percent of patients were homozygous for this allele, which results in 28% lower bioavailability compared to wild-type individuals. Thirty-seven percent of patients were heterozygous with a 22% lower bioavailability compared to wild-type individuals, giving a total of 89% of patients having lower bioavailability. Since the proportion of patients expected to have lower bioavailability is so high, a general dose increment for the entire South African population is not unreasonable. This is especially so when one considers the fact that current rifampicin doses are already on the lower end of optimal exposure. Furthermore, we have shown (via a contingency table) that a rifampicin C<sub>max</sub> less than 8 mg/L is associated with a significantly higher probability of a positive sputum culture result after 8 weeks of treatment. This agrees with previous recommendations of a minimum C<sub>max</sub> of 8 mg/L (Peloquin 2002). The clinical impact of the *SLCO1B1 rs4149032* polymorphism

has thus been successfully evaluated and provides justification for further studies investigating higher doses in South African patients.

The current study has some limitations. Firstly, the limited number of patients with both genotypic and pharmacokinetic data (57 patients) may have led to a compromised ability to detect the effect of (the less common) *PXR*, *CAR*, *ABCB1*, or indeed other *SLCO1B1* polymorphisms on rifampicin pharmacokinetics. Secondly, whilst the effect on the 2 month culture conversion status has been shown, patients were not followed up to treatment completion (6 months duration) hence the effect on final patient outcome and relapse rates (needing 1-2 years of follow up) is as yet unknown. Lastly, it has recently been shown that rifampicin is metabolised by AADAC (Nakajima *et al.* 2011). Even more recently, SNPs leading to decreased activity of AADAC have been identified (Shimizu *et al.* 2012). Unfortunately these recent publications were not available at the time of commencing the study (2009). Thus further work should include determination of the effect of new (and the few currently reported) AADAC SNPs on AADAC's deacetylation activity, determination of the frequency of these SNPs in the African population, and ultimately their effect of rifampicin pharmacokinetics and pharmacodynamics.

### **13.2 A Description of the Pharmacokinetics of the First-line Antitubercular Drugs In South African Patients**

The pharmacokinetics of rifampicin was adequately described using a transit compartment absorption model with first order elimination. Apart from *SLCO1B1 rs4149032*, other factors affecting interindividual variability were body weight, sex (males had a higher volume of distribution and a shorter MTT) which are consistent with other studies (Nijland *et al.* 2006; McIlleron *et al.* 2012). The administered dose was also a significant covariate (higher dose

resulting in shorter MTT). The high variability in absorption in previous studies (Wilkins *et al.* 2008) was also present in this dataset.

A novel finding is that pyrazinamide undergoes parallel first order and mixed order (saturable) elimination. This means that calls for higher doses of more than double the current doses per unit weight, which have been based on linear pharmacokinetics (Gumbo *et al.* 2009) should be interpreted with caution before proceeding to clinical trials because they can result in an underprediction of the AUC by at least 25%. The current model which is more physiological, can be used to accurately predict the required dose to attain the desired exposure.

Bimodality in isoniazid pharmacokinetics, most likely due to NAT-2 polymorphism was observed in the clearance of our study population, as expected. A limitation is that financial resources did not permit genotyping for NAT-2 polymorphisms in order to test for their significance on isoniazid pharmacokinetics. However, this would not have been a meaningful contribution to the field since this is a well studied pharmacogenetic phenomenon. We nevertheless managed to build on previous models and experience in a similar population to be able to accurately estimate isoniazid exposure for subsequent evaluation of its contribution to bacillary decline. Ethambutol pharmacokinetics was adequately described using a one compartment model with first order elimination and a transit compartment absorption model.

After successful development and evaluation, the models for rifampicin, isoniazid, pyrazinamide and ethambutol were used to obtain summary measures of drug exposure (AUC and C<sub>max</sub>) for use in subsequent analyses.

### **13.3 Based on the PK-PD Index of AUC/MIC, is Ofloxacin Drug Exposure Adequate in South African Patients with MDR-TB?**

Fluoroquinolones are a key drug class in treatment of MDR-TB characterised by resistance to isoniazid and rifampicin. The fluoroquinolone routinely administered to South African patients was ofloxacin. Our results show the inadequacy of ofloxacin drug exposure when compared to the PK-PD target,  $AUC/MIC > 100$ . Based on the current 800 mg dose the PTA expectation was only 0.45. Considering the observed range of MICs in our South African patients, our findings support the replacement of ofloxacin with moxifloxacin or levofloxacin. In addition, the higher MICs in Cape Town compared to Durban patients warrants further investigation to determine possible causes, e.g. different *M. tuberculosis* strains. This is also the first study to describe ofloxacin pharmacokinetics through quantifying clearance via the glomerular filtration and extra-glomerular filtration routes. The model gives an accurate description of the effect of renal function on the various clearance pathways of ofloxacin. Therefore the model can be used to investigate the effect of renal function on ofloxacin exposure and can be used to make the necessary dose adjustments.

### **13.4 How can one quantitatively analyze pharmacodynamic data comprised of days to positivity in automated liquid culture from patients on TB treatment?**

A semi-mechanistic model has been developed which can quantitatively describe the trend of increasing days to a positive MGIT culture result for weekly sputum samples collected from patients on treatment for TB. The main parts of this novel time to event pharmacodynamic model are biexponential decline in the bacillary load in patients' sputum, a logistic model describing the growth of the bacteria in liquid culture, followed by a hazard model to describe the probability of a positive test result during incubation related to the amount of oxygen that has been consumed by the mycobacteria. The model has the advantage of being able to capture changes in the MGIT culture growth kinetics of *M. tuberculosis* with time on

treatment, in addition to describing the decline in bacillary load in sputum. *M. tuberculosis*-specific covariates such as strain, subtype or genotype can be investigated as covariates on the growth kinetics. Similarly, patient-specific covariates can be investigated on various parts of the biexponential model (i.e. intercepts and slopes). An example of such implementation was the presence of lung cavitation which was found to result in a higher baseline amount of bacteria that are killed slowly. HIV status was not significant on the model parameters, in agreement with previous studies which did not find HIV to affect sputum culture conversion rates (Brindle *et al.* 1993; Senkoro *et al.* 2010; Brust *et al.* 2011). Comparison of the effects of one drug regimen vs. another on the model parameters could be tested within the model. Thus the model has several potential applications, but should be confirmed using data from other studies.

A limitation of this model is that there is currently a lack of *in vitro* data to support some of the findings relating to the growth kinetics of the bacteria in MGIT culture. Further work should involve investigation of the metabolic activity and growth rates of bacteria isolated from patients during the course of treatment since these factors may change with time.

Although there is a decontamination process in the MGIT process whereby antibiotics are used to kill non tubercular micro-organisms, there is no guarantee that the culture is pure *M. tuberculosis*. There is now emerging technology to identify and quantify many types of bacteria (including various types of mycobacteria) based on molecular methods (16S rRNA and/or rDNA gene sequencing) in combination with molecular databases (Woo *et al.*, 2008; Woo *et al.*, 2011). In future, such methods may be useful in determining the actual amount of *M. tuberculosis* in a sputum specimen and would be more specific than the MGIT system.

### 13.5 What is the Effect of Individual Drug Exposure on Disease Regression and Treatment Outcome in South African Patients with TB?

The effects of drug AUCs and Cmaxs for rifampicin, isoniazid, pyrazinamide and ethambutol from the developed pharmacokinetic models were investigated in the time to event model above. Rifampicin AUC significantly influenced the alpha slope of the biexponential decay model. Since this population of bacteria was eradicated after a just over a week, the may not be of significance for treatment that lasts up to 6 months (including 2 months of the intensive phase). Failure to find an effect of any drug on the beta slopes may be due to the limited sample size. In addition, non-linear relationships would be more physiological than linear relationships. However, there was not sufficient power to discern an effect of drug exposure within the range of our study which investigated a single dose per unit of body weight and included only 73 patients. Reliable identification of sigmoidal relationships needs administration of a wider range of doses in larger studies.

An important finding based on the data-driven CART analysis method is the result that a rifampicin Cmax less than 8.55 mg/L is associated with a significantly higher probability of sputum positive results at the end of the intensive phase of TB treatment (2 months). The 2 month sputum result is a recognized surrogate of sterilizing activity and relapse (Mitchison 1993; Burman 2003). With a high proportion of South Africans carrying the *SLCO1B1* rs4149032 polymorphism which leads to decreased bioavailability and a higher chance of a Cmax less than 8.55 mg/L, this calls for re-evaluation of the dose of rifampicin in this population. Interestingly, this cut-off Cmax is in strong agreement with a previously recommended minimum value of 8 mg/L implemented in a well-recognized therapeutic drug monitoring lab (Peloquin 2002). The finding that rifampicin Cmax and not AUC is predictive of 2 month culture status is also in strong agreement with a previous study (Pasipanodya *et*

*al.* 2012) which found the AUC to be more predictive of long term treatment outcome rather than 2 month solid culture results.

A limitation of this study is that MICs for the first-line drugs were not available. AUC/MIC ratios would be an even more reliable PK-PD index rather than the AUC alone. Thus further work in clinical studies should include MIC determination and thereafter identify AUC/MIC cut-offs for the first-line drugs, thereby enabling a PTA analysis similar to what has been done for ofloxacin to be carried out. The paradoxical effect of ethambutol C<sub>max</sub> on 2 month outcomes needs confirmation in further studies.

### **13.6 How should one design a study to investigate the sterilizing activity of one drug regimen vs. another?**

Part of this thesis points towards a need for higher drug doses or alternative drug regimens, and this means further studies in this direction are required. Existing methods of conducting clinical trials take a long time, require many patients and are costly. This work explored an alternative approach that can be used test different study designs and thus identify an efficient method of comparison of drug regimens. NLME is a recognized powerful tool for analysis of data comprised of serial sputum CFU counts towards the investigation of new drug regimens (Davies *et al.* 2006). There is therefore a need to optimally design clinical trials which intend to use NLME as the primary method of data analysis. Based on a literature model of decline in CFU counts with time on treatment (Rustomjee *et al.* 2008), clinical trial simulation was used to describe the optimum study design. A parallel study with 2 arms would need a total of 250 patients to achieve a study power of 80% to detect a difference of 25% between the 2 regimens in the kill rate of the mycobacteria that are killed slowly (thus a measure of sterilizing activity) when NLME is to be used for analysis. The number of patients is

drastically reduced if a crossover study is used. The modeling approach to the data analysis can overcome some of the pitfalls of a crossover study, especially pharmacokinetic carryover. Pharmacodynamic carryover can also be taken into account if the underlying biology is known (which may not always be the case). Importantly, clinical trial simulation revealed that longer study duration does not perpetually result in more information, at least from a perspective of determining a difference in kill rates in sputum. Based on the current kill rates, a study duration of 35-42 days is ideal. Further work in the application of clinical trial simulation in the field of TB should include investigation of pharmacodynamic carryover i.e. the effect of one drug regimen irreversibly altering the susceptibility of the bacteria to the subsequent regimen. In addition, since a model for disease regression based on liquid culture result has been developed, a similar approach can be investigated using this measurement instead of CFU counting on solid media. Unfortunately, difficulties in simulating an event in a survival model implemented in the version of Pharsight Trial Simulator<sup>®</sup> 2.2.1 that was used made this impossible. Future studies may test the clinical trial simulation approach using other analytical tools (e.g. NONMEM, but would have extremely long times for the complex model) or future versions of the currently available Pharsight Trial Simulator<sup>®</sup> 2.2.1 that may have addressed this problem.

### **13.7 CONCLUSION**

In conclusion, all 6 objectives were achieved. To our knowledge, this thesis reports for the first time: 1) novel pharmacogenetic determinants of rifampicin pharmacokinetics in South Africans, possibly explaining lower 2 month sputum conversion rates found in Africans compared with non-Africans, 2) parallel first order and mixed order elimination of pyrazinamide enabling more accurate prediction of the exposure following administration of higher doses, 3) inadequacy of ofloxacin drug exposure in relation to MICs in South African



patients and supporting replacement of the drug with other fluoroquinolones, 4) a novel semi-mechanistic time to event pharmacodynamic model to analyse data from liquid culture, 5) the influence of rifampicin C<sub>max</sub> on 2 month sputum liquid culture conversion, and 6) the exploration of ideal study designs for phase II clinical trials using clinical trial simulation, and demonstrates the potentially powerful application of clinical trial design using NLME methods for analysis of data from serial sputum samples.

Based on the work in this thesis, further studies should involve large pharmacogenetic studies across different populations to confirm the effect of *SLCO1B1* and *AADAC* polymorphisms on rifampicin pharmacokinetics and pharmacodynamics. Further work should also involve optimization of fluoroquinolone doses (levofloxacin and moxifloxacin in particular) with more extensive descriptions of the distribution of MICs in the relevant target populations. *In vitro* studies to determine mycobacterial growth kinetics in MGIT culture, as well as confirmation of our TTD model in patients on other drug regimens would also be suitable future work. Based on these more mechanistic TTD models, identifying the sampling design most powerful for evaluating sterilizing activity of drugs can be determined in further studies. The ideal study design, data analysis methods and markers of sterilizing activity need further investigation in clinical studies to validate our suggestions based on clinical trial simulation. Datasets from more recent studies could be used to build models which can then be used for clinical trial simulation to confirm the most efficient designs to test regimens based on their sterilizing activity.

## REFERENCES

- Abe, T., M. Unno, T. Onogawa and T. Tokui (2001). "[Molecular identification of organic anion transporter oatp/LST family]." Tanpakushitsu Kakusan Koso **46**(5): 612-20.
- Acocella, G. (1978). "Clinical pharmacokinetics of rifampicin." Clin Pharmacokinet **3**(2): 108-27.
- Acocella, G. (1983). "Pharmacokinetics and metabolism of rifampin in humans." Rev Infect Dis **5 Suppl 3**: S428-32.
- Agrawal, S., K. J. Kaur, I. Singh, S. R. Bhade, C. L. Kaul and R. Panchagnula (2002). "Assessment of bioequivalence of rifampicin, isoniazid and pyrazinamide in a four drug fixed dose combination with separate formulations at the same dose levels." Int J Pharm **233**(1-2): 169-77.
- Agrawal, S., I. Singh, K. J. Kaur, S. Bhade, C. L. Kaul and R. Panchagnula (2004). "Bioequivalence trials of rifampicin containing formulations: extrinsic and intrinsic factors in the absorption of rifampicin." Pharmacol Res **50**(3): 317-27.
- Agrawal, S., I. Singh, K. J. Kaur, S. R. Bhade, C. L. Kaul and R. Panchagnula (2004). "Comparative bioavailability of rifampicin, isoniazid and pyrazinamide from a four drug fixed dose combination with separate formulations at the same dose levels." Int J Pharm **276**(1-2): 41-9.
- Ahmad, S., G. F. Araj, P. K. Akbar, E. Fares, T. D. Chugh and A. S. Mustafa (2000). "Characterization of rpoB mutations in rifampin-resistant Mycobacterium tuberculosis isolates from the Middle East." Diagn Microbiol Infect Dis **38**(4): 227-32.
- Ahn, J. E., M. O. Karlsson, A. Dunne and T. M. Ludden (2008). "Likelihood based approaches to handling data below the quantification limit using NONMEM VI." J Pharmacokinet Pharmacodyn **35**(4): 401-21.
- Ait Moussa, L., C. E. Khassouani, B. Hue, M. Jana, B. Begaud and R. Soulaymani (2002). "Determination of the acetylator phenotype in Moroccan tuberculosis patients using isoniazid as metabolic probe." Int J Clin Pharmacol Ther **40**(12): 548-53.
- Anderson, B. J. and N. H. Holford (2008). "Mechanism-based concepts of size and maturity in pharmacokinetics." Annu Rev Pharmacol Toxicol **48**: 303-32.
- Andries, K., P. Verhasselt, J. Guillemont, H. W. Gohlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis and V. Jarlier (2005). "A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis." Science **307**(5707): 223-7.
- Angeby, K. A., P. Jureen, C. G. Giske, E. Chryssanthou, E. Sturegard, M. Nordvall, A. G. Johansson, J. Werngren, G. Kahlmeter, S. E. Hoffner and T. Schon (2010). "Wild-type MIC distributions of four fluoroquinolones active against Mycobacterium tuberculosis in relation to current critical concentrations and available pharmacokinetic and pharmacodynamic data." J Antimicrob Chemother **65**(5): 946-52.

Annadurai, G., S. Rajesh Babu and V. Srinivasamoorthy, R (2000). "Development of mathematical models (Logistic, Gompertz, and Richards models) describing the growth pattern of pseudomonas putida (NICM 2174)." Bioprocess Engineering **23**: 607-612.

Anonymous (1921). "Decline of Tuberculosis in New York City." Am J Public Health (N Y) **11**(8): 746-8.

Anonymous (1976). "A comparative study of daily followed by twice- or once-weekly regimens of ethambutol and rifampicin in the retreatment of patients with pulmonary tuberculosis: second report." Tubercle **57**(2): 105-13.

Back, D. J. and S. M. Rogers (1987). "Review: first-pass metabolism by the gastrointestinal mucosa." Aliment Pharmacol Ther **1**(5): 339-57.

Barrett, J. C., B. Fry, J. Maller and M. J. Daly (2005). "Haploview: analysis and visualization of LD and haplotype maps." Bioinformatics **21**(2): 263-5.

Beal, S. L. (2001). "Ways to fit a PK model with some data below the quantification limit." J Pharmacokinet Pharmacodyn **28**(5): 481-504.

Bliven-Sizemore, E., A. Kashuba, S. Malone, M. Weiner, E. L. Nuermberger, W. Burman, S. E. Dorman and K. E. Dooley (2011). CYP3A Induction by Rifampin and Rifapentine: Which Drug and Dose Does it Best? 4th International Workshop on Clinical Pharmacology of Tuberculosis Drugs, Chicago, USA.

Blodgett, R. J. (2008). "Mathematical treatment of plates with colony counts outside the acceptable range." Food Microbiol **25**(1): 92-8.

Blumberg, H. M., W. J. Burman, R. E. Chaisson, C. L. Daley, S. C. Etkind, L. N. Friedman, P. Fujiwara, M. Grzemska, P. C. Hopewell, M. D. Iseman, R. M. Jasmer, V. Koppaka, R. I. Menzies, R. J. O'Brien, R. R. Reves, L. B. Reichman, P. M. Simone, J. R. Starke and A. A. Vernon (2003). "American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis." Am J Respir Crit Care Med **167**(4): 603-62.

Bock, N. N., T. R. Sterling, C. D. Hamilton, C. Pachucki, Y. C. Wang, D. S. Conwell, A. Mosher, M. Samuels and A. Vernon (2002). "A prospective, randomized, double-blind study of the tolerability of rifapentine 600, 900, and 1,200 mg plus isoniazid in the continuation phase of tuberculosis treatment." Am J Respir Crit Care Med **165**(11): 1526-30.

Boehme, C. C., P. Nabeta, D. Hillemann, M. P. Nicol, S. Shenai, F. Krapp, J. Allen, R. Tahirli, R. Blakemore, R. Rustomjee, A. Milovic, M. Jones, S. M. O'Brien, D. H. Persing, S. Ruesch-Gerdes, E. Gotuzzo, C. Rodrigues, D. Alland and M. D. Perkins (2010). "Rapid molecular detection of tuberculosis and rifampin resistance." N Engl J Med **363**(11): 1005-15.

Boman, G. (1974). "Serum concentration and half-life of rifampicin after simultaneous oral administration of aminosalicylic acid or isoniazid." Eur J Clin Pharmacol **7**(3): 217-25.

Bonate, P. L. (2000). "Clinical trial simulation in drug development." Pharm Res **17**(3): 252-6.

Bonicke, R. and W. Reif (1953). "[Enzymatic inactivation of isonicotinic acid hydrazide in human and animal organism]." Naunyn Schmiedebergs Arch Exp Pathol Pharmacol **220**(4): 321-3.

Botha, F. J., F. A. Sirgel, D. P. Parkin, B. W. van de Wal, P. R. Donald and D. A. Mitchison (1996). "Early bactericidal activity of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater) in patients with pulmonary tuberculosis." S Afr Med J **86**(2): 155-8.

Brieman, L., J. Friedman, R. A. Olshen and C. J. Stone (1984). Classification and regression trees. Monterey, CA, Wadsworth and Brooks.

Brindle, R., J. Odhiambo and D. Mitchison (2001). "Serial counts of Mycobacterium tuberculosis in sputum as surrogate markers of the sterilising activity of rifampicin and pyrazinamide in treating pulmonary tuberculosis." BMC Pulm Med **1**: 2.

Brindle, R. J., P. P. Nunn, W. Githui, B. W. Allen, S. Gathua and P. Waiyaki (1993). "Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis." Am Rev Respir Dis **147**(4): 958-61.

British Medical Research Council (1948). "STREPTOMYCIN treatment of pulmonary tuberculosis." Br Med J **2**(4582): 769-82.

Brugger, S. D., C. Baumberger, M. Jost, W. Jenni, U. Brugger and K. Muhlemann (2012). "Automated counting of bacterial colony forming units on agar plates." PLoS One **7**(3): e33695.

Brust, J. C., M. Lygizos, K. Chaiyachati, M. Scott, T. L. van der Merwe, A. P. Moll, X. Li, M. Loveday, S. A. Bamber, U. G. Lalloo, G. H. Friedland, N. S. Shah and N. R. Gandhi (2011). "Culture conversion among HIV co-infected multidrug-resistant tuberculosis patients in Tugela Ferry, South Africa." PLoS One **6**(1): e15841.

Budha, N. R., R. B. Lee, J. G. Hurdle, R. E. Lee and B. Meibohm (2009). "A simple in vitro PK/PD model system to determine time-kill curves of drugs against Mycobacteria." Tuberculosis (Edinb) **89**(5): 378-85.

Burk, O., K. A. Arnold, A. Geick, H. Tegude and M. Eichelbaum (2005). "A role for constitutive androstane receptor in the regulation of human intestinal MDR1 expression." Biol Chem **386**(6): 503-13.

Burman, W. J. (2003). "The hunt for the elusive surrogate marker of sterilizing activity in tuberculosis treatment." Am J Respir Crit Care Med **167**(10): 1299-301.

Burman, W. J., S. Goldberg, J. L. Johnson, G. Muzanye, M. Engle, A. W. Mosher, S. Choudhri, C. L. Daley, S. S. Munsiff, Z. Zhao, A. Vernon and R. E. Chaisson (2006). "Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis." Am J Respir Crit Care Med **174**(3): 331-8.

Campagna, M., A. Calix and G. Hauser (1954). "Observations on the combined use of pyrazinamide (Aldinamide) and isoniazid in the treatment of pulmonary tuberculosis; a clinical study." Am Rev Tuberc **69**(3): 334-50.

Cascorbi, I., T. Gerloff, A. John, C. Meisel, S. Hoffmeyer, M. Schwab, E. Schaeffeler, M. Eichelbaum, U. Brinkmann and I. Roots (2001). "Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects." Clin Pharmacol Ther **69**(3): 169-74.

Chagnac, A., M. Herman, B. Zingerman, A. Erman, B. Rozen-Zvi, J. Hirsh and U. Gafter (2008). "Obesity-induced glomerular hyperfiltration: its involvement in the pathogenesis of tubular sodium reabsorption." Nephrol Dial Transplant **23**(12): 3946-52.

Chan, C. Y., C. Au-Yeang, W. W. Yew, M. Hui and A. F. Cheng (2001). "Postantibiotic effects of antituberculosis agents alone and in combination." Antimicrob Agents Chemother **45**(12): 3631-4.

Chan, S. L., W. W. Yew, W. K. Ma, D. J. Girling, V. R. Aber, D. Felmingham, B. W. Allen and D. A. Mitchison (1992). "The early bactericidal activity of rifabutin measured by sputum viable counts in Hong Kong patients with pulmonary tuberculosis." Tuber Lung Dis **73**(1): 33-8.

Chang, C. L., T. S. Park, S. H. Oh, H. H. Kim, E. Y. Lee, H. C. Son and C. M. Kim (2002). "Reduction of contamination of mycobacterial growth indicator tubes with a modified antimicrobial combination." J Clin Microbiol **40**(10): 3845-7.

Chang, K. C., C. C. Leung, W. W. Yew, S. C. Ho and C. M. Tam (2004). "A nested case-control study on treatment-related risk factors for early relapse of tuberculosis." Am J Respir Crit Care Med **170**(10): 1124-30.

Chen, B., J. H. Li, Y. M. Xu, J. Wang and X. M. Cao (2006). "The influence of NAT2 genotypes on the plasma concentration of isoniazid and acetylisoniazid in Chinese pulmonary tuberculosis patients." Clin Chim Acta **365**(1-2): 104-8.

Chen, J. and K. Raymond (2006). "Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor." Ann Clin Microbiol Antimicrob **5**: 3.

Chew, W. K., R. M. Lasaitis, F. A. Schio and G. L. Gilbert (1998). "Clinical evaluation of the Mycobacteria Growth Indicator Tube (MGIT) compared with radiometric (Bactec) and solid media for isolation of Mycobacterium species." J Med Microbiol **47**(9): 821-7.

Chideya, S., C. A. Winston, C. A. Peloquin, W. Z. Bradford, P. C. Hopewell, C. D. Wells, A. L. Reingold, T. A. Kenyon, T. L. Moeti and J. W. Tappero (2009). "Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana." Clin Infect Dis **48**(12): 1685-94.

Chien, H. P., M. C. Yu, M. H. Wu, T. P. Lin and K. T. Luh (2000). "Comparison of the BACTEC MGIT 960 with Lowenstein-Jensen medium for recovery of mycobacteria from clinical specimens." Int J Tuberc Lung Dis **4**(9): 866-70.

Chigutsa, E., J. S. Van Der Valt, P. Denti, P. Smith and H. McIlleron (2009). A mixture model showing bimodal rifampicin absorption and clearance: Do drug transporter polymorphisms play a role? 5th International Conference on Pharmaceutical and Pharmacological Sciences, Potchefstroom, South Africa.

Cho, H. J., W. J. Koh, Y. J. Ryu, C. S. Ki, M. H. Nam, J. W. Kim and S. Y. Lee (2007). "Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis." Tuberculosis (Edinb) **87**(6): 551-6.

Christensen, E. (2007). "Methodology of superiority vs. equivalence trials and non-inferiority trials." J Hepatol **46**(5): 947-54.

Cohen, T., B. Sommers and M. Murray (2003). "The effect of drug resistance on the fitness of Mycobacterium tuberculosis." Lancet Infect Dis **3**(1): 13-21.

Coletsos, P. J. (1953). "[Development of resistance of Mycobacterium tuberculosis in pulmonary tuberculosis during isoniazid therapy]." Rev Tuberc **17**(1-2): 75-89.

Comas, I., S. Borrell, A. Roetzer, G. Rose, B. Malla, M. Kato-Maeda, J. Galagan, S. Niemann and S. Gagneux (2011). "Whole-genome sequencing of rifampicin-resistant Mycobacterium tuberculosis strains identifies compensatory mutations in RNA polymerase genes." Nat Genet **44**(1): 106-10.

Corbett, E. L., C. J. Watt, N. Walker, D. Maher, B. G. Williams, M. C. Raviglione and C. Dye (2003). "The growing burden of tuberculosis: global trends and interactions with the HIV epidemic." Arch Intern Med **163**(9): 1009-21.

Cowley, D., D. Govender, B. February, M. Wolfe, L. Steyn, J. Evans, R. J. Wilkinson and M. P. Nicol (2008). "Recent and rapid emergence of W-Beijing strains of Mycobacterium tuberculosis in Cape Town, South Africa." Clin Infect Dis **47**(10): 1252-9.

Crofton, J. and D. A. Mitchison (1948). "Streptomycin resistance in pulmonary tuberculosis." Br Med J **2**(4588): 1009-15.

David, H. L. (1970). "Probability distribution of drug-resistant mutants in unselected populations of Mycobacterium tuberculosis." Appl Microbiol **20**(5): 810-4.

Davies, G. R., R. Brindle, S. H. Khoo and L. J. Aarons (2006). "Use of nonlinear mixed-effects analysis for improved precision of early pharmacodynamic measures in tuberculosis treatment." Antimicrob Agents Chemother **50**(9): 3154-6.

Davies, G. R., S. H. Khoo and L. J. Aarons (2006). "Optimal sampling strategies for early pharmacodynamic measures in tuberculosis." J Antimicrob Chemother **58**(3): 594-600.

de Jager, P. and R. van Altena (2002). "Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis." Int J Tuberc Lung Dis **6**(7): 622-7.

Delaude, A., M. F. Jorda and G. Moreau (1962). "[Action of ethambutol on experimental tuberculosis in the guinea pig]." Rev Tuberc Pneumol (Paris) **26**: 519-24.

DeMets, D. L. and R. M. Califf (2011). "A historical perspective on clinical trials innovation and leadership: where have the academics gone?" Jama **305**(7): 713-4.

Den Boon, S., E. D. Bateman, D. A. Enarson, M. W. Borgdorff, S. Verver, C. J. Lombard, E. Iruen, N. Beyers and N. W. White (2005). "Development and evaluation of a new chest

radiograph reading and recording system for epidemiological surveys of tuberculosis and lung disease." Int J Tuberc Lung Dis **9**(10): 1088-96.

Desjardin, L. E., M. D. Perkins, K. Wolski, S. Haun, L. Teixeira, Y. Chen, J. L. Johnson, J. J. Ellner, R. Dietze, J. Bates, M. D. Cave and K. D. Eisenach (1999). "Measurement of sputum *Mycobacterium tuberculosis* messenger RNA as a surrogate for response to chemotherapy." Am J Respir Crit Care Med **160**(1): 203-10.

Dhillon, J., D. B. Lowrie and D. A. Mitchison (2004). "Mycobacterium tuberculosis from chronic murine infections that grows in liquid but not on solid medium." BMC Infect Dis **4**: 51.

Diacon, A. H., R. Dawson, M. Hanekom, K. Narunsky, S. J. Maritz, A. Venter, P. R. Donald, C. van Niekerk, K. Whitney, D. J. Rouse, M. W. Laurenzi, A. M. Ginsberg and M. K. Spigelman (2010). "Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients." Antimicrob Agents Chemother **54**(8): 3402-7.

Diacon, A. H., J. S. Maritz, A. Venter, P. D. van Helden, K. Andries, D. F. McNeeley and P. R. Donald (2010). "Time to detection of the growth of *Mycobacterium tuberculosis* in MGIT 960 for determining the early bactericidal activity of antituberculosis agents." Eur J Clin Microbiol Infect Dis **29**(12): 1561-5.

Diacon, A. H., J. S. Maritz, A. Venter, P. D. van Helden, R. Dawson and P. R. Donald (2011). "Time to liquid culture positivity can substitute for colony counting on agar plates in early bactericidal activity studies of antituberculosis agents." Clin Microbiol Infect **18**(7): 711-7.

Diacon, A. H., R. F. Patientia, A. Venter, P. D. van Helden, P. J. Smith, H. McIlleron, J. S. Maritz and P. R. Donald (2007). "Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears." Antimicrob Agents Chemother **51**(8): 2994-6.

Diacon, A. H., A. Pym, M. Grobusch, R. Patientia, R. Rustomjee, L. Page-Shipp, C. Pistorius, R. Krause, M. Bogoshi, G. Churchyard, A. Venter, J. Allen, J. C. Palomino, T. De Marez, R. P. van Heeswijk, N. Lounis, P. Meyvisch, J. Verbeeck, W. Parys, K. de Beule, K. Andries and D. F. Mc Neeley (2009). "The diarylquinoline TMC207 for multidrug-resistant tuberculosis." N Engl J Med **360**(23): 2397-405.

Dickinson, J. M., V. R. Aber and D. A. Mitchison (1977). "Bactericidal activity of streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide alone and in combination against *Mycobacterium Tuberculosis*." Am Rev Respir Dis **116**(4): 627-35.

Dickinson, J. M. and D. A. Mitchison (1981). "Experimental models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis." Am Rev Respir Dis **123**(4 Pt 1): 367-71.

Dietze, R., D. J. Hadad, B. McGee, L. P. Molino, E. L. Maciel, C. A. Peloquin, D. F. Johnson, S. M. Debanne, K. Eisenach, W. H. Boom, M. Palaci and J. L. Johnson (2008). "Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis." Am J Respir Crit Care Med **178**(11): 1180-5.

Domingue, G., J. W. Costerton and M. R. Brown (1996). "Bacterial doubling time modulates the effects of opsonisation and available iron upon interactions between *Staphylococcus aureus* and human neutrophils." FEMS Immunol Med Microbiol **16**(3-4): 223-8.

Donald, P. R., D. P. Parkin, H. I. Seifart, H. S. Schaaf, P. D. van Helden, C. J. Werely, F. A. Sirgel, A. Venter and J. S. Maritz (2007). "The influence of dose and N-acetyltransferase-2 (NAT2) genotype and phenotype on the pharmacokinetics and pharmacodynamics of isoniazid." Eur J Clin Pharmacol **63**(7): 633-9.

Donald, P. R., F. A. Sirgel, F. J. Botha, H. I. Seifart, D. P. Parkin, M. L. Vandenplas, B. W. Van de Wal, J. S. Maritz and D. A. Mitchison (1997). "The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis." Am J Respir Crit Care Med **156**(3 Pt 1): 895-900.

Dorman, S. E., J. L. Johnson, S. Goldberg, G. Muzanye, N. Padayatchi, L. Bozeman, C. M. Heilig, J. Bernardo, S. Choudhri, J. H. Grosset, E. Guy, P. Guyadeen, M. C. Leus, G. Maltas, D. Menzies, E. L. Nuermberger, M. Villarino, A. Vernon and R. E. Chaisson (2009). "Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis." Am J Respir Crit Care Med **180**(3): 273-80.

DrugBank (2012). Isoniazid, <http://www.drugbank.ca/drugs/DB00951>, Accessed 19 October 2012.

Drusano, G. L., S. L. Preston, C. Hardalo, R. Hare, C. Banfield, D. Andes, O. Vesga and W. A. Craig (2001). "Use of preclinical data for selection of a phase II/III dose for evernimicin and identification of a preclinical MIC breakpoint." Antimicrob Agents Chemother **45**(1): 13-22.

Duong, D. A., T. H. Nguyen, T. N. Nguyen, V. H. Dai, T. M. Dang, S. K. Vo, D. A. Do, V. V. Nguyen, H. D. Nguyen, N. S. Dinh, J. Farrar and M. Caws (2009). "Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with high-level fluoroquinolone resistance in Vietnam." Antimicrob Agents Chemother **53**(11): 4835-9.

Ellard, G. A. (1969). "Absorption, metabolism and excretion of pyrazinamide in man." Tubercle **50**(2): 144-58.

Ellard, G. A. and P. T. Gammon (1976). "Pharmacokinetics of isoniazid metabolism in man." J Pharmacokinet Biopharm **4**(2): 83-113.

Espinal, M. A., S. J. Kim, P. G. Suarez, K. M. Kam, A. G. Khomenko, G. B. Migliori, J. Baez, A. Kochi, C. Dye and M. C. Ravigliione (2000). "Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries." Jama **283**(19): 2537-45.

Evans, D. A. and T. A. White (1964). "Human Acetylation Polymorphism." J Lab Clin Med **63**: 394-403.

Faber, K. N., M. Muller and P. L. Jansen (2003). "Drug transport proteins in the liver." Adv Drug Deliv Rev **55**(1): 107-24.

Falagas, M. E., P. I. Rafailidis and E. S. Rosmarakis (2007). "Arrhythmias associated with fluoroquinolone therapy." Int J Antimicrob Agents **29**(4): 374-9.



FDA. (2010). "Pharmacometrics at FDA." Retrieved 17 December 2012, from <http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm167032.htm>.

Fretland, A. J., M. A. Leff, M. A. Doll and D. W. Hein (2001). "Functional characterization of human N-acetyltransferase 2 (NAT2) single nucleotide polymorphisms." *Pharmacogenetics* **11**(3): 207-15.

Garcia-Rodriguez, J. F., H. Alvarez-Diaz, M. V. Lorenzo-Garcia, A. Marino-Callejo, A. Fernandez-Rial and P. Sesma-Sanchez (2011). "Extrapulmonary tuberculosis: epidemiology and risk factors." *Enferm Infecc Microbiol Clin* **29**(7): 502-9.

Gibson, A. M., N. Bratchell and T. A. Roberts (1987). "The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry." *J Appl Bacteriol* **62**(6): 479-90.

Gieschke, R. and J. L. Steimer (2000). "Pharmacometrics: modelling and simulation tools to improve decision making in clinical drug development." *Eur J Drug Metab Pharmacokinet* **25**(1): 49-58.

Gill, W. P., N. S. Harik, M. R. Whiddon, R. P. Liao, J. E. Mittler and D. R. Sherman (2009). "A replication clock for Mycobacterium tuberculosis." *Nat Med* **15**(2): 211-4.

Gillespie, S. H., O. J. Billington, A. Breathnach and T. D. McHugh (2002). "Multiple drug-resistant Mycobacterium tuberculosis: evidence for changing fitness following passage through human hosts." *Microb Drug Resist* **8**(4): 273-9.

Gillespie, S. H., R. D. Gosling and B. M. Charalambous (2002). "A reiterative method for calculating the early bactericidal activity of antituberculosis drugs." *Am J Respir Crit Care Med* **166**(1): 31-5.

Gillespie, S. H., R. D. Gosling, L. Uiso, N. E. Sam, E. G. Kanduma and T. D. McHugh (2005). "Early bactericidal activity of a moxifloxacin and isoniazid combination in smear-positive pulmonary tuberculosis." *J Antimicrob Chemother* **56**(6): 1169-71.

Ginsberg, A. M., M. W. Laurenzi, D. J. Rouse, K. D. Whitney and M. K. Spigelman (2009). "Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects." *Antimicrob Agents Chemother* **53**(9): 3720-5.

Ginsberg, A. M. and M. Spigelman (2007). "Challenges in tuberculosis drug research and development." *Nat Med* **13**(3): 290-4.

Ginsburg, A. S., J. H. Grosset and W. R. Bishai (2003). "Fluoroquinolones, tuberculosis, and resistance." *Lancet Infect Dis* **3**(7): 432-42.

Ginsburg, A. S., N. Hooper, N. Parrish, K. E. Dooley, S. E. Dorman, J. Booth, M. Diener-West, W. G. Merz, W. R. Bishai and T. R. Sterling (2003). "Fluoroquinolone resistance in patients with newly diagnosed tuberculosis." *Clin Infect Dis* **37**(11): 1448-52.

Ginsburg, A. S., S. C. Woolwine, N. Hooper, W. H. Benjamin, Jr., W. R. Bishai, S. E. Dorman and T. R. Sterling (2003). "The rapid development of fluoroquinolone resistance in M. tuberculosis." *N Engl J Med* **349**(20): 1977-8.

Girard, P. (2005). "Clinical trial simulation: a tool for understanding study failures and preventing them." Basic Clin Pharmacol Toxicol **96**(3): 228-34.

Gler, M. T., V. Skripconoka, E. Sanchez-Garavito, H. Xiao, J. L. Cabrera-Rivero, D. E. Vargas-Vasquez, M. Gao, M. Awad, S. K. Park, T. S. Shim, G. Y. Suh, M. Danilovits, H. Ogata, A. Kurve, J. Chang, K. Suzuki, T. Tupasi, W. J. Koh, B. Seaworth, L. J. Geiter and C. D. Wells (2012). "Delamanid for multidrug-resistant pulmonary tuberculosis." N Engl J Med **366**(23): 2151-60.

Gomberg-Maitland, M., L. Frison and J. L. Halperin (2003). "Active-control clinical trials to establish equivalence or noninferiority: methodological and statistical concepts linked to quality." Am Heart J **146**(3): 398-403.

Gomez, F. D. (1952). "[First clinical results obtained with isoniazid in the treatment of pulmonary tuberculosis; preliminary note]." Hoja Tisiol **12**(2): 111-25.

Gomez, J. E. and J. D. McKinney (2004). "M. tuberculosis persistence, latency, and drug tolerance." Tuberculosis (Edinb) **84**(1-2): 29-44.

Gompertz, B. (1825). "On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies." Philos. Trans. R. Soc. London **115**: 513-585.

Gosling, R. D., L. O. Uiso, N. E. Sam, E. Bongard, E. G. Kanduma, M. Nyindo, R. W. Morris and S. H. Gillespie (2003). "The bactericidal activity of moxifloxacin in patients with pulmonary tuberculosis." Am J Respir Crit Care Med **168**(11): 1342-5.

Goto, M., S. Masuda, H. Saito, S. Uemoto, T. Kiuchi, K. Tanaka and K. Inui (2002). "C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation." Pharmacogenetics **12**(6): 451-7.

Grant, S. S., B. B. Kaufmann, N. S. Chand, N. Haseley and D. T. Hung (2012). "Eradication of bacterial persisters with antibiotic-generated hydroxyl radicals." Proc Natl Acad Sci U S A **109**(30): 12147-52.

Grosset, J. (1978). "The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy." Bull Int Union Tuberc **53**(1): 5-12.

Gumbo, T. (2010). "New susceptibility breakpoints for first-line antituberculosis drugs based on antimicrobial pharmacokinetic/pharmacodynamic science and population pharmacokinetic variability." Antimicrob Agents Chemother **54**(4): 1484-91.

Gumbo, T., C. S. Dona, C. Meek and R. Leff (2009). "Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel in vitro model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs." Antimicrob Agents Chemother **53**(8): 3197-204.

Gumbo, T., A. Louie, M. R. Deziel, W. Liu, L. M. Parsons, M. Salfinger and G. L. Drusano (2007). "Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin." Antimicrob Agents Chemother **51**(11): 3781-8.

Gumbo, T., A. Louie, M. R. Deziel, L. M. Parsons, M. Salfinger and G. L. Drusano (2004). "Selection of a moxifloxacin dose that suppresses drug resistance in Mycobacterium tuberculosis, by use of an in vitro pharmacodynamic infection model and mathematical modeling." J Infect Dis **190**(9): 1642-51.

Gumbo, T., A. Louie, W. Liu, P. G. Ambrose, S. M. Bhavnani, D. Brown and G. L. Drusano (2007). "Isoniazid's bactericidal activity ceases because of the emergence of resistance, not depletion of Mycobacterium tuberculosis in the log phase of growth." J Infect Dis **195**(2): 194-201.

Gumbo, T., A. Louie, W. Liu, D. Brown, P. G. Ambrose, S. M. Bhavnani and G. L. Drusano (2007). "Isoniazid bactericidal activity and resistance emergence: integrating pharmacodynamics and pharmacogenomics to predict efficacy in different ethnic populations." Antimicrob Agents Chemother **51**(7): 2329-36.

Gurumurthy, P., G. Ramachandran, A. K. Hemanth Kumar, S. Rajasekaran, C. Padmapriyadarsini, S. Swaminathan, S. Bhagavathy, P. Venkatesan, L. Sekar, A. Mahilmaran, N. Ravichandran and P. Paramesh (2004). "Decreased bioavailability of rifampin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease." Antimicrob Agents Chemother **48**(11): 4473-5.

Gutierrez, M. C., S. Brisse, R. Brosch, M. Fabre, B. Omais, M. Marmiesse, P. Supply and V. Vincent (2005). "Ancient origin and gene mosaicism of the progenitor of Mycobacterium tuberculosis." PLoS Pathog **1**(1 e5): 0055-0061.

Gyselen, A., L. Verbist, J. Cossemans, L. M. Lacquet and E. Vandenberg (1968). "Rifampin and ethambutol in the retreatment of advanced pulmonary tuberculosis." Am Rev Respir Dis **98**(6): 933-41.

Haas, C. J., A. Zink, E. Molnar, U. Szeimies, U. Reischl, A. Marcsik, Y. Ardagna, O. Dutour, G. Palfi and A. G. Nerlich (2000). "Molecular evidence for different stages of tuberculosis in ancient bone samples from Hungary." Am J Phys Anthropol **113**(3): 293-304.

Haas, C. N. and B. Heller (1988). "Averaging of TNTC counts." Appl Environ Microbiol **54**(8): 2069-72.

Hall, R. G., 2nd, M. A. Swancutt, C. Meek, R. D. Leff and T. Gumbo (2012). "Ethambutol pharmacokinetic variability is linked to body mass in overweight, obese, and extremely obese people." Antimicrob Agents Chemother **56**(3): 1502-7.

Hand, D. J. and R. J. Till (2001). "A Simple Generalisation of the Area Under the ROC Curve for Multiple Class Classification Problems." Machine Learning **45**: 171-186.

Health and Medical Publishing Group (2010). "South African medicines formulary, 9th ed." 312-313.

Heifets, L. B., M. D. Iseman and P. J. Lindholm-Levy (1988). "Combinations of rifampin or rifabutine plus ethambutol against Mycobacterium avium complex. Bactericidal synergistic, and bacteriostatic additive or synergistic effects." Am Rev Respir Dis **137**(3): 711-5.

Hershkovitz, I., H. D. Donoghue, D. E. Minnikin, G. S. Besra, O. Y. Lee, A. M. Gernaey, E. Galili, V. Eshed, C. L. Greenblatt, E. Lemma, G. K. Bar-Gal and M. Spigelman (2008).

"Detection and molecular characterization of 9,000-year-old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean." PLoS One **3**(10): e3426.

Heym, B., P. M. Alzari, N. Honore and S. T. Cole (1995). "Missense mutations in the catalase-peroxidase gene, *katG*, are associated with isoniazid resistance in *Mycobacterium tuberculosis*." Mol Microbiol **15**(2): 235-45.

Hoffmeyer, S., O. Burk, O. von Richter, H. P. Arnold, J. Brockmoller, A. John, I. Cascorbi, T. Gerloff, I. Roots, M. Eichelbaum and U. Brinkmann (2000). "Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo." Proc Natl Acad Sci U S A **97**(7): 3473-8.

Holford, N. H. (1996). "A size standard for pharmacokinetics." Clin Pharmacokinet **30**(5): 329-32.

Holford, N., S. C. Ma and B. A. Ploeger (2010). "Clinical trial simulation: a review." Clin Pharmacol Ther **88**(2): 166-82.

Holford, N. H., H. C. Kimko, J. P. Monteleone and C. C. Peck (2000). "Simulation of clinical trials." Annu Rev Pharmacol Toxicol **40**: 209-34.

Holtz, T. H., M. Sternberg, S. Kammerer, K. F. Laserson, V. Riekstina, E. Zarovska, V. Skripconoka, C. D. Wells and V. Leimane (2006). "Time to sputum culture conversion in multidrug-resistant tuberculosis: predictors and relationship to treatment outcome." Ann Intern Med **144**(9): 650-9.

Hu, Y., A. R. Coates and D. A. Mitchison (2003). "Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*." Antimicrob Agents Chemother **47**(2): 653-7.

Huang, Y. S., H. D. Chern, W. J. Su, J. C. Wu, S. C. Chang, C. H. Chiang, F. Y. Chang and S. D. Lee (2003). "Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis." Hepatology **37**(4): 924-30.

Huang, Y. S., H. D. Chern, W. J. Su, J. C. Wu, S. L. Lai, S. Y. Yang, F. Y. Chang and S. D. Lee (2002). "Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis." Hepatology **35**(4): 883-9.

Ibrahim, M., C. Truffot-Pernot, K. Andries, V. Jarlier and N. Veziris (2009). "Sterilizing activity of R207910 (TMC207)-containing regimens in the murine model of tuberculosis." Am J Respir Crit Care Med **180**(6): 553-7.

International Hapmap Project (2010). [http://hapmap.ncbi.nlm.nih.gov/cgi-372perl/gbrowse/hapmap28\\_B36/](http://hapmap.ncbi.nlm.nih.gov/cgi-372perl/gbrowse/hapmap28_B36/). Accessed 12 December 2010., International Hapmap Project.

Jamis-Dow, C. A., A. G. Katki, J. M. Collins and R. W. Klecker (1997). "Rifampin and rifabutin and their metabolism by human liver esterases." Xenobiotica **27**(10): 1015-24.

Janmahasatian, S., S. B. Duffull, S. Ash, L. C. Ward, N. M. Byrne and B. Green (2005). "Quantification of lean bodyweight." Clin Pharmacokinet **44**(10): 1051-65.

Janmahasatian, S., S. B. Duffull, A. Chagnac, C. M. Kirkpatrick and B. Green (2008). "Lean body mass normalizes the effect of obesity on renal function." Br J Clin Pharmacol **65**(6): 964-5.

Janssen-Ortho (2006). "Floxin Product Monograph. Janssen-Ortho. Toronto, Canada." 17.

Jayaram, R., S. Gaonkar, P. Kaur, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharat, R. K. Shandil, E. Kantharaj and V. Balasubramanian (2003). "Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis." Antimicrob Agents Chemother **47**(7): 2118-24.

Jayaram, R., R. K. Shandil, S. Gaonkar, P. Kaur, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharath, E. Kantharaj and V. Balasubramanian (2004). "Isoniazid pharmacokinetics-pharmacodynamics in an aerosol infection model of tuberculosis." Antimicrob Agents Chemother **48**(8): 2951-7.

Jeanes, C. W., A. G. Jessamine and L. Eidus (1972). "Treatment of chronic drug-resistant pulmonary tuberculosis with rifampin and ethambutol." Can Med Assoc J **106**(8): 884-8.

Jesudason, D. R. and P. Clifton (2011). "Interpreting different measures of glomerular filtration rate in obesity and weight loss: pitfalls for the clinician." Int J Obes (Lond).

Ji, B., N. Lounis, C. Truffot-Pernot and J. Grosset (1995). "In vitro and in vivo activities of levofloxacin against Mycobacterium tuberculosis." Antimicrob Agents Chemother **39**(6): 1341-4.

Jindani, A., V. R. Aber, E. A. Edwards and D. A. Mitchison (1980). "The early bactericidal activity of drugs in patients with pulmonary tuberculosis." Am Rev Respir Dis **121**(6): 939-49.

Jindani, A., C. J. Dore and D. A. Mitchison (2003). "Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days." Am J Respir Crit Care Med **167**(10): 1348-54.

Joel, F., M. Leong, F. Eum, L. E. Via and C. E. Barry, 3rd (2012). Pathology of Tuberculosis in the Human Lung. A Color Atlas of Comparative Pathology of Pulmonary Tuberculosis. F. J. Leong, V. Dartois and T. Dick, CRC Press.

Johnsson, K., D. S. King and P. G. Schultz (1995). "Studies on the Mechanism of Action of Isoniazid and Ethionamide in the Chemotherapy of Tuberculosis." J. Am. Chem. Soc. **117**: 5009-5010.

Joloba, M. L., J. L. Johnson, A. Namale, A. Morrissey, A. E. Assegghai, R. D. Mugerwa, J. J. Ellner and K. D. Eisenach (2000). "Quantitative sputum bacillary load during rifampin-containing short course chemotherapy in human immunodeficiency virus-infected and non-infected adults with pulmonary tuberculosis." Int J Tuberc Lung Dis **4**(6): 528-36.

Jones, B., P. Jarvis, J. A. Lewis and A. F. Ebbutt (1996). "Trials to assess equivalence: the importance of rigorous methods." Bmj **313**(7048): 36-9.

Jonsson, S., A. Davidse, J. Wilkins, J. S. Van der Walt, U. S. Simonsson, M. O. Karlsson, P. Smith and H. McIlleron (2011). "Population pharmacokinetics of ethambutol in South African tuberculosis patients." Antimicrob Agents Chemother **55**(9): 4230-7.

Kang, D., J. B. Schwartz and D. Verotta (2004). "A sample size computation method for non-linear mixed effects models with applications to pharmacokinetics models." Stat Med **23**(16): 2551-66.

Karlsson, K. E., E. L. Plan and M. O. Karlsson (2011). "Performance of three estimation methods in repeated time-to-event modeling." Aaps J **13**(1): 83-91.

Karlsson, M. O., S. L. Beal and L. B. Sheiner (1995). "Three new residual error models for population PK/PD analyses." J Pharmacokinet Biopharm **23**(6): 651-72.

Kim, R. B. (2003). "Organic anion-transporting polypeptide (OATP) transporter family and drug disposition." Eur J Clin Invest **33 Suppl 2**: 1-5.

Kim, R. B., B. F. Leake, E. F. Choo, G. K. Dresser, S. V. Kubba, U. I. Schwarz, A. Taylor, H. G. Xie, J. McKinsey, S. Zhou, L. B. Lan, J. D. Schuetz, E. G. Schuetz and G. R. Wilkinson (2001). "Identification of functionally variant MDR1 alleles among European Americans and African Americans." Clin Pharmacol Ther **70**(2): 189-99.

Kiser, J. J., R. Zhu, D. Z. D'Argenio, M. F. Cotton, R. Bobat, G. D. McSherry, S. A. Madhi, V. J. Carey, H. I. Seifart, C. J. Werely and C. V. Fletcher (2012). "Isoniazid pharmacokinetics, pharmacodynamics, and dosing in South African infants." Ther Drug Monit **34**(4): 446-51.

Kita, T., Y. Tanigawara, S. Chikazawa, H. Hatanaka, T. Sakaeda, F. Komada, S. Iwakawa and K. Okumura (2001). "N-Acetyltransferase2 genotype correlated with isoniazid acetylation in Japanese tuberculous patients." Biol Pharm Bull **24**(5): 544-9.

Kjellsson, M. C., L. E. Via, A. Goh, D. Weiner, K. M. Low, S. Kern, G. Pillai, C. E. Barry, 3rd and V. Dartois (2012). "Pharmacokinetic evaluation of the penetration of antituberculosis agents in rabbit pulmonary lesions." Antimicrob Agents Chemother **56**(1): 446-57.

Kocagoz, T., C. J. Hackbarth, I. Unsal, E. Y. Rosenberg, H. Nikaido and H. F. Chambers (1996). "Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra." Antimicrob Agents Chemother **40**(8): 1768-74.

Konig, J., Y. Cui, A. T. Nies and D. Keppler (2000). "Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide." J Biol Chem **275**(30): 23161-8.

Konig, J., Y. Cui, A. T. Nies and D. Keppler (2000). "A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane." Am J Physiol Gastrointest Liver Physiol **278**(1): G156-64.

Lacroix, C., T. P. Hoang, J. Nouveau, C. Guyonnaud, G. Laine, H. Duwoos and O. Lafont (1989). "Pharmacokinetics of pyrazinamide and its metabolites in healthy subjects." Eur J Clin Pharmacol **36**(4): 395-400.

Lamba, J., V. Lamba, S. Strom, R. Venkataramanan and E. Schuetz (2008). "Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression." Drug Metab Dispos **36**(1): 169-81.

Lee, C. S., D. C. Brater, J. G. Gambertoglio and L. Z. Benet (1980). "Disposition kinetics of ethambutol in man." J Pharmacokinet Biopharm **8**(4): 335-46.

Lee, C. S., J. G. Gambertoglio, D. C. Brater and L. Z. Benet (1977). "Kinetics of oral ethambutol in the normal subject." Clin Pharmacol Ther **22**(5 Pt 1): 615-21.

Lei, B., C. J. Wei and S. C. Tu (2000). "Action mechanism of antitubercular isoniazid. Activation by Mycobacterium tuberculosis KatG, isolation, and characterization of inhA inhibitor." J Biol Chem **275**(4): 2520-6.

Lemon, S. C., J. Roy, M. A. Clark, P. D. Friedmann and W. Rakowski (2003). "Classification and regression tree analysis in public health: methodological review and comparison with logistic regression." Ann Behav Med **26**(3): 172-81.

Li, A. P., M. K. Reith, A. Rasmussen, J. C. Gorski, S. D. Hall, L. Xu, D. L. Kaminski and L. K. Cheng (1997). "Primary human hepatocytes as a tool for the evaluation of structure-activity relationship in cytochrome P450 induction potential of xenobiotics: evaluation of rifampin, rifapentine and rifabutin." Chem Biol Interact **107**(1-2): 17-30.

Lillebaek, T., A. Dirksen, I. Baess, B. Strunge, V. O. Thomsen and A. B. Andersen (2002). "Molecular evidence of endogenous reactivation of Mycobacterium tuberculosis after 33 years of latent infection." J Infect Dis **185**(3): 401-4.

Lockwood, P., W. Ewy, D. Hermann and N. Holford (2006). "Application of clinical trial simulation to compare proof-of-concept study designs for drugs with a slow onset of effect; an example in Alzheimer's disease." Pharm Res **23**(9): 2050-9.

Lode, H., G. Hoffken, P. Olschewski, B. Sievers, A. Kirch, K. Borner and P. Koeppe (1987). "Pharmacokinetics of ofloxacin after parenteral and oral administration." Antimicrob Agents Chemother **31**(9): 1338-42.

Long, M. W., D. E. Snider, Jr. and L. S. Farer (1979). "U.S. Public Health Service Cooperative trial of three rifampin-isoniazid regimens in treatment of pulmonary tuberculosis." Am Rev Respir Dis **119**(6): 879-94.

Loos, U., E. Musch, J. C. Jensen, G. Mikus, H. K. Schwabe and M. Eichelbaum (1985). "Pharmacokinetics of oral and intravenous rifampicin during chronic administration." Klin Wochenschr **63**(23): 1205-11.

Louw, G. E., R. M. Warren, N. C. Gey van Pittius, R. Leon, A. Jimenez, R. Hernandez-Pando, C. R. McEvoy, M. Grobbelaar, M. Murray, P. D. van Helden and T. C. Victor (2011). "Rifampicin reduces susceptibility to ofloxacin in rifampicin-resistant Mycobacterium tuberculosis through efflux." Am J Respir Crit Care Med **184**(2): 269-76.

Lubomirov, R., J. di Iulio, A. Fayet, S. Colombo, R. Martinez, C. Marzolini, H. Furrer, P. Vernazza, A. Calmy, M. Cavassini, B. Ledergerber, K. Rentsch, P. Descombes, T. Buclin, L. A. Decosterd, C. Csajka and A. Telenti (2010). "ADME pharmacogenetics: investigation of

the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir." Pharmacogenet Genomics **20**(4): 217-30.

Lucchesi, M., A. Bancalè, G. Pallotta and A. Termine (1962). "[1st clinical observations in the tuberculosis field with rifamycin SV administered by venous perfusion]." Ann Ist Carlo Forlanini **22**: 441-69.

Mackness, G. B. and N. Smith (1952). "The action of isoniazid (isonicotinic acid hydrazide) on intracellular tubercle bacilli." Am Rev Tuberc **66**(2): 125-33.

Maggi, N., C. R. Pasqualucci, R. Ballotta and P. Sensi (1966). "Rifampicin: a new orally active rifamycin." Chemotherapy **11**(5): 285-92.

Maisnier-Patin, S. and D. I. Andersson (2004). "Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution." Res Microbiol **155**(5): 360-9.

Mariam, D. H., Y. Mengistu, S. E. Hoffner and D. I. Andersson (2004). "Effect of rpoB mutations conferring rifampin resistance on fitness of Mycobacterium tuberculosis." Antimicrob Agents Chemother **48**(4): 1289-94.

Mason, E. and D. W. Russell (1971). "Isoniazid acetylation rates (phenotypes) of patients being treated for tuberculosis." Bull World Health Organ **45**(5): 617-24.

Matimba, A., J. Del-Favero, C. Van Broeckhoven and C. Masimirembwa (2009). "Novel variants of major drug-metabolising enzyme genes in diverse African populations and their predicted functional effects." Hum Genomics **3**(2): 169-90.

McIlleron, H., J. Norman, T. P. Kanyok, P. B. Fourie, J. Horton and P. J. Smith (2007). "Elevated gatifloxacin and reduced rifampicin concentrations in a single-dose interaction study amongst healthy volunteers." J Antimicrob Chemother **60**(6): 1398-401.

McIlleron, H., R. Rustomjee, M. Vahedi, T. Mthiyane, P. Denti, C. Connolly, W. Rida, A. Pym, P. J. Smith and P. C. Onyebujoh (2012). "Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: implications for international dosing guidelines." Antimicrob Agents Chemother **56**(6): 3232-8.

McIlleron, H., P. Wash, A. Burger, J. Norman, P. I. Folb and P. Smith (2006). "Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients." Antimicrob Agents Chemother **50**(4): 1170-7.

McIlleron, H., M. Willemsse, C. J. Werely, G. D. Hussey, H. S. Schaaf, P. J. Smith and P. R. Donald (2009). "Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines." Clin Infect Dis **48**(11): 1547-53.

Mehta, J. B., A. Dutt, L. Harvill and K. M. Mathews (1991). "Epidemiology of extrapulmonary tuberculosis. A comparative analysis with pre-AIDS era." Chest **99**(5): 1134-8.

Merck (2001). "Zinamide Summary of Product Characteristics." Merck Sharp and Dohme.



Meredith, S. A., P. J. Smith, J. Norman and L. Wiesner (2012). "An LC-MS/MS method for the determination of ofloxacin in 20µl human plasma." J Pharm Biomed Anal **58**: 177-81.

Migliori, G. B., C. Lange, E. Girardi, R. Centis, G. Besozzi, K. Kliiman, L. R. Codecasa, A. Spanevello and D. M. Cirillo (2008). "Fluoroquinolones: are they essential to treat multidrug-resistant tuberculosis?" Eur Respir J **31**(4): 904-5.

Miller, R., W. Ewy, B. W. Corrigan, D. Ouellet, D. Hermann, K. G. Kowalski, P. Lockwood, J. R. Koup, S. Donevan, A. El-Kattan, C. S. Li, J. L. Werth, D. E. Feltner and R. L. Lalonde (2005). "How modeling and simulation have enhanced decision making in new drug development." J Pharmacokinet Pharmacodyn **32**(2): 185-97.

Mitchison, D. A. (1993). "Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months." Am Rev Respir Dis **147**(4): 1062-3.

Mitchison, D. A. (2000). "Role of individual drugs in the chemotherapy of tuberculosis." Int J Tuberc Lung Dis **4**(9): 796-806.

Mitnick, C. D., B. McGee and C. A. Peloquin (2009). "Tuberculosis pharmacotherapy: strategies to optimize patient care." Expert Opin Pharmacother **10**(3): 381-401.

Moriya, Y., T. Nakamura, M. Horinouchi, T. Sakaeda, T. Tamura, N. Aoyama, T. Shirakawa, A. Gotoh, S. Fujimoto, M. Matsuo, M. Kasuga and K. Okumura (2002). "Effects of polymorphisms of MDR1, MRP1, and MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects." Biol Pharm Bull **25**(10): 1356-9.

Mouton, J. W., M. N. Dudley, O. Cars, H. Derendorf and G. L. Drusano (2005). "Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update." J Antimicrob Chemother **55**(5): 601-7.

Nakajima, A., T. Fukami, Y. Kobayashi, A. Watanabe, M. Nakajima and T. Yokoi (2011). "Human arylacetamide deacetylase is responsible for deacetylation of rifamycins: rifampicin, rifabutin, and rifapentine." Biochem Pharmacol **82**(11): 1747-56.

Nakamura, T., T. Sakaeda, M. Horinouchi, T. Tamura, N. Aoyama, T. Shirakawa, M. Matsuo, M. Kasuga and K. Okumura (2002). "Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects." Clin Pharmacol Ther **71**(4): 297-303.

NCBI (2010). Reference SNP Cluster Report. Available [http://www.ncbi.nlm.nih.gov/projects/SNP/snp\\_ref.cgi?rs=4149032](http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4149032). Accessed 12 December 2010., National Center for Biotechnology Information.

Niemi, M., E. Schaeffeler, T. Lang, M. F. Fromm, M. Neuvonen, C. Kyrklund, J. T. Backman, R. Kerb, M. Schwab, P. J. Neuvonen, M. Eichelbaum and K. T. Kivisto (2004). "High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1)." Pharmacogenetics **14**(7): 429-40.

Nijland, H. M., R. Ruslami, J. E. Stalenhoef, E. J. Nelwan, B. Alisjahbana, R. H. Nelwan, A. J. van der Ven, H. Danusantoso, R. E. Aarnoutse and R. van Crevel (2006). "Exposure to

rifampicin is strongly reduced in patients with tuberculosis and type 2 diabetes." Clin Infect Dis **43**(7): 848-54.

Nozawa, T., M. Nakajima, I. Tamai, K. Noda, J. Nezu, Y. Sai, A. Tsuji and T. Yokoi (2002). "Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis." J Pharmacol Exp Ther **302**(2): 804-13.

Nuermberger, E. L., T. Yoshimatsu, S. Tyagi, R. J. O'Brien, A. N. Vernon, R. E. Chaisson, W. R. Bishai and J. H. Grosset (2004). "Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis." Am J Respir Crit Care Med **169**(3): 421-6.

Nuermberger, E. L., T. Yoshimatsu, S. Tyagi, K. Williams, I. Rosenthal, R. J. O'Brien, A. A. Vernon, R. E. Chaisson, W. R. Bishai and J. H. Grosset (2004). "Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis." Am J Respir Crit Care Med **170**(10): 1131-4.

Nunn, A. J., P. P. Phillips and S. H. Gillespie (2008). "Design issues in pivotal drug trials for drug sensitive tuberculosis (TB)." Tuberculosis (Edinb) **88 Suppl 1**: S85-92.

O'Brien, R. J. (2002). "Studies of the early bactericidal activity of new drugs for tuberculosis: a help or a hindrance to antituberculosis drug development?" Am J Respir Crit Care Med **166**(1): 3-4.

O'Sullivan, D. M., T. D. McHugh and S. H. Gillespie (2010). "Mapping the fitness of Mycobacterium tuberculosis strains: a complex picture." J Med Microbiol **59**(Pt 12): 1533-5.

Ohno, M., I. Yamaguchi, I. Yamamoto, T. Fukuda, S. Yokota, R. Maekura, M. Ito, Y. Yamamoto, T. Ogura, K. Maeda, K. Komuta, T. Igarashi and J. Azuma (2000). "Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity." Int J Tuberc Lung Dis **4**(3): 256-61.

Orenstein, E. W., S. Basu, N. S. Shah, J. R. Andrews, G. H. Friedland, A. P. Moll, N. R. Gandhi and A. P. Galvani (2009). "Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis." Lancet Infect Dis **9**(3): 153-61.

Otu, J., M. Antonio, Y. B. Cheung, S. Donkor, B. C. De Jong, T. Corrah and R. A. Adegbola (2008). "Comparative evaluation of BACTEC MGIT 960 with BACTEC 9000 MB and LJ for isolation of mycobacteria in The Gambia." J Infect Dev Ctries **2**(3): 200-5.

Paramasivan, C. N., S. Sulochana, G. Kubendiran, P. Venkatesan and D. A. Mitchison (2005). "Bactericidal action of gatifloxacin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of Mycobacterium tuberculosis." Antimicrob Agents Chemother **49**(2): 627-31.

Parkin, D. P., S. Vandenplas, F. J. Botha, M. L. Vandenplas, H. I. Seifart, P. D. van Helden, B. J. van der Walt, P. R. Donald and P. P. van Jaarsveld (1997). "Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis." Am J Respir Crit Care Med **155**(5): 1717-22.

Pasipanodya, J. G. and T. Gumbo (2010). "Clinical and toxicodynamic evidence that high-dose pyrazinamide is not more hepatotoxic than the low doses currently used." Antimicrob Agents Chemother **54**(7): 2847-54.

Pasipanodya, J. G., H. McIlleron, A. Burger, P. Wash, P. Smith and T. Gumbo (2012). PK/PD of first line antituberculosis drugs and concentrations associated with optimal efficacy in combination therapy regimens in adult patients. 5th International Workshop on Clinical Pharmacology of TB drugs, San Francisco, USA, Virology Education.

Pathak, S., J. A. Awuh, N. A. Leversen, T. H. Flo and B. Asjo (2012). "Counting mycobacteria in infected human cells and mouse tissue: a comparison between qPCR and CFU." PLoS One **7**(4): e34931.

Peloquin, C. A. (1996). "Therapeutic drug monitoring of the antimycobacterial drugs." Clin Lab Med **16**(3): 717-29.

Peloquin, C. A. (2002). "Therapeutic drug monitoring in the treatment of tuberculosis." Drugs **62**(15): 2169-83.

Peloquin, C. A., A. E. Bulpitt, G. S. Jaresko, R. W. Jelliffe, J. M. Childs and D. E. Nix (1999). "Pharmacokinetics of ethambutol under fasting conditions, with food, and with antacids." Antimicrob Agents Chemother **43**(3): 568-72.

Peloquin, C. A., A. E. Bulpitt, G. S. Jaresko, R. W. Jelliffe, G. T. James and D. E. Nix (1998). "Pharmacokinetics of pyrazinamide under fasting conditions, with food, and with antacids." Pharmacotherapy **18**(6): 1205-11.

Peloquin, C. A., D. J. Hadad, L. P. Molino, M. Palaci, W. H. Boom, R. Dietze and J. L. Johnson (2008). "Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis." Antimicrob Agents Chemother **52**(3): 852-7.

Peloquin, C. A., G. S. Jaresko, C. L. Yong, A. C. Keung, A. E. Bulpitt and R. W. Jelliffe (1997). "Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide." Antimicrob Agents Chemother **41**(12): 2670-9.

Peloquin, C. A., R. Namdar, A. A. Dodge and D. E. Nix (1999). "Pharmacokinetics of isoniazid under fasting conditions, with food, and with antacids." Int J Tuberc Lung Dis **3**(8): 703-10.

Peloquin, C. A., R. Namdar, M. D. Singleton and D. E. Nix (1999). "Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids." Chest **115**(1): 12-8.

Perlman, D. C., Y. Segal, S. Rosenkranz, P. M. Rainey, R. P. Remmel, N. Salomon, R. Hafner and C. A. Peloquin (2005). "The clinical pharmacokinetics of rifampin and ethambutol in HIV-infected persons with tuberculosis." Clin Infect Dis **41**(11): 1638-47.

Perrin, F. M., M. C. Lipman, T. D. McHugh and S. H. Gillespie (2007). "Biomarkers of treatment response in clinical trials of novel antituberculosis agents." Lancet Infect Dis **7**(7): 481-90.

Perrin, F. M., N. Woodward, P. P. Phillips, T. D. McHugh, A. J. Nunn, M. C. Lipman and S. H. Gillespie (2010). "Radiological cavitation, sputum mycobacterial load and treatment response in pulmonary tuberculosis." Int J Tuberc Lung Dis **14**(12): 1596-602.

Pheiffer, C., N. M. Carroll, N. Beyers, P. Donald, K. Duncan, P. Uys and P. van Helden (2008). "Time to detection of Mycobacterium tuberculosis in BACTEC systems as a viable alternative to colony counting." Int J Tuberc Lung Dis **12**(7): 792-8.

Philip, R. (1928). "An Address on THE CAUSES OF THE DECLINE IN TUBERCULOSIS MORTALITY." Br Med J **1**(3512): 701-6.

Piaggio, G., D. R. Elbourne, D. G. Altman, S. J. Pocock and S. J. Evans (2006). "Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement." Jama **295**(10): 1152-60.

Pillai, G., P. B. Fourie, N. Padayatchi, P. C. Onyebujoh, H. McIlleron, P. J. Smith and G. Gabriels (1999). "Recent bioequivalence studies on fixed-dose combination anti-tuberculosis drug formulations available on the global market." Int J Tuberc Lung Dis **3**(11 Suppl 3): S309-16; discussion S317-21.

Pillay, M. and A. W. Sturm (2007). "Evolution of the extensively drug-resistant F15/LAM4/KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa." Clin Infect Dis **45**(11): 1409-14.

Pletz, M. W., A. De Roux, A. Roth, K. H. Neumann, H. Mauch and H. Lode (2004). "Early bactericidal activity of moxifloxacin in treatment of pulmonary tuberculosis: a prospective, randomized study." Antimicrob Agents Chemother **48**(3): 780-2.

Possuelo, L. G., J. A. Castelan, T. C. de Brito, A. W. Ribeiro, P. I. Cafrune, P. D. Picon, A. R. Santos, R. L. Teixeira, T. S. Gregianini, M. H. Hutz, M. L. Rossetti and A. Zaha (2008). "Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil." Eur J Clin Pharmacol **64**(7): 673-81.

Prakash, J., T. Velpandian, J. N. Pande and S. K. Gupta (2003). "Serum Rifampicin Levels in Patients with Tuberculosis : Effect of P-Glycoprotein and CYP3A4 Blockers on its Absorption." Clin Drug Investig **23**(7): 463-72.

Prasad, R., S. K. Verma, S. Sahai, S. Kumar and A. Jain (2006). "Efficacy and safety of kanamycin, ethionamide, PAS and cycloserine in multidrug-resistant pulmonary tuberculosis patients." Indian J Chest Dis Allied Sci **48**(3): 183-6.

Pray, L. (2008). "DNA replication and causes of mutation." Nature Education **1**(1).

Preston, S. L., P. J. Piliero, J. A. Bilello, D. S. Stein, W. T. Symonds and G. L. Drusano (2003). "In vitro-in vivo model for evaluating the antiviral activity of amprenavir in combination with ritonavir administered at 600 and 100 milligrams, respectively, every 12 hours." Antimicrob Agents Chemother **47**(11): 3393-9.

Putt, M. E. and B. Ravina (2002). "Randomized, placebo-controlled, parallel group versus crossover study designs for the study of dementia in Parkinson's disease." Control Clin Trials **23**(2): 111-26.

R\_Development\_Core\_Team (2008). "A language and environment for statistical computing. R Foundation for Statistical Computing."

Rae, J. M., M. D. Johnson, M. E. Lippman and D. A. Flockhart (2001). "Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays." J Pharmacol Exp Ther **299**(3): 849-57.

Rajman, I. (2008). "PK/PD modelling and simulations: utility in drug development." Drug Discov Today **13**(7-8): 341-6.

Raviglione, M. C., C. Dye, S. Schmidt and A. Kochi (1997). "Assessment of worldwide tuberculosis control. WHO Global Surveillance and Monitoring Project." Lancet **350**(9078): 624-9.

Rey-Jurado, E., G. Tudo, S. Borrell, F. Alcaide, P. Coll, M. Espanol, N. Martin-Casabona, V. Mick, M. Montemayor, R. Moure, M. Salvado, E. Vicente and J. Gonzalez-Martin (2011). "Impaired fitness of Mycobacterium tuberculosis resistant isolates in a cell culture model of murine macrophages." J Antimicrob Chemother **66**(10): 2277-80.

Ruslami, R., H. M. Nijland, B. Alisjahbana, I. Parwati, R. van Crevel and R. E. Aarnoutse (2007). "Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients." Antimicrob Agents Chemother **51**(7): 2546-51.

Rustomjee, R., C. Lienhardt, T. Kanyok, G. R. Davies, J. Levin, T. Mthiyane, C. Reddy, A. W. Sturm, F. A. Sirgel, J. Allen, D. J. Coleman, B. Fourie and D. A. Mitchison (2008). "A Phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis." Int J Tuberc Lung Dis **12**(2): 128-38.

Sabbagh, A., A. Langaney, P. Darlu, N. Gerard, R. Krishnamoorthy and E. S. Poloni (2008). "Worldwide distribution of NAT2 diversity: implications for NAT2 evolutionary history." BMC Genet **9**: 21.

Saito, H. (1998). "[Laboratory media for the cultivation of tubercle bacillus]." Kekkaku **73**(5): 329-37.

Sakaeda, T., T. Nakamura, M. Horinouchi, M. Kakumoto, N. Ohmoto, T. Sakai, Y. Morita, T. Tamura, N. Aoyama, M. Hirai, M. Kasuga and K. Okumura (2001). "MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects." Pharm Res **18**(10): 1400-4.

Sakula, A. (1982). "Robert Koch: centenary of the discovery of the tubercle bacillus, 1882." Thorax **37**(4): 246-51.

Sandoz (2008). Rimactazid summary of product characteristics, section 5.2. Denmark.

Sanofi-Aventis (2007). Rifadin® (Rifampin) product monograph. Version 3.0:15. Canada.

Sato, K., Y. Inoue, T. Fujii, H. Aoyama and S. Mitsuhashi (1986). "Antibacterial activity of ofloxacin and its mode of action." Infection **14 Suppl 4**: S226-30.

Savic, R. M., D. M. Jonker, T. Kerbusch and M. O. Karlsson (2007). "Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies." J Pharmacokinet Pharmacodyn **34**(5): 711-26.

Schaaf, H. S., D. P. Parkin, H. I. Seifart, C. J. Werely, P. B. Hesselning, P. D. van Helden, J. S. Maritz and P. R. Donald (2005). "Isoniazid pharmacokinetics in children treated for respiratory tuberculosis." Arch Dis Child **90**(6): 614-8.

Schaeffeler, E., M. Eichelbaum, U. Brinkmann, A. Penger, S. Asante-Poku, U. M. Zanger and M. Schwab (2001). "Frequency of C3435T polymorphism of MDR1 gene in African people." Lancet **358**(9279): 383-4.

Schatz, A., E. Bugie and S. A. Waksman (1944). "Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria." Proc Soc Exp Biol Med **55**(1): 66-69.

Schentag, J. J., A. K. Meagher and A. Forrest (2003). "Fluoroquinolone AUC break points and the link to bacterial killing rates. Part 1: In vitro and animal models." Ann Pharmacother **37**(9): 1287-98.

Schentag, J. J., A. K. Meagher and A. Forrest (2003). "Fluoroquinolone AUC break points and the link to bacterial killing rates. Part 2: human trials." Ann Pharmacother **37**(10): 1478-88.

Schuetz, E. G., A. H. Schinkel, M. V. Relling and J. D. Schuetz (1996). "P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans." Proc Natl Acad Sci U S A **93**(9): 4001-5.

Selkon, J. B., S. Devadatta, K. G. Kulkarni, D. A. Mitchison, A. S. Narayana, C. N. Nair and K. Ramachandran (1964). "The Emergence of Isoniazid-Resistant Cultures in Patients with Pulmonary Tuberculosis During Treatment with Isoniazid Alone or Isoniazid Plus Pas." Bull World Health Organ **31**: 273-94.

Senkoro, M., S. G. Mfinanga and O. Morkve (2010). "Smear microscopy and culture conversion rates among smear positive pulmonary tuberculosis patients by HIV status in Dar es Salaam, Tanzania." BMC Infect Dis **10**: 210.

Sensi, P. (1983). "History of the development of rifampin." Rev Infect Dis **5 Suppl 3**: S402-6.

Shandil, R. K., R. Jayaram, P. Kaur, S. Gaonkar, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharath and V. Balasubramanian (2007). "Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against Mycobacterium tuberculosis: evaluation of in vitro and pharmacodynamic indices that best predict in vivo efficacy." Antimicrob Agents Chemother **51**(2): 576-82.

Shean, K. P., P. A. Willcox, S. N. Siwendu, K. F. Laserson, L. Gross, S. Kammerer, C. D. Wells and T. H. Holtz (2008). "Treatment outcome and follow-up of multidrug-resistant tuberculosis patients, West Coast/Winelands, South Africa, 1992-2002." Int J Tuberc Lung Dis **12**(10): 1182-9.

Sheiner, L. B. and S. L. Beal (1980). "Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data." J Pharmacokinet Biopharm **8**(6): 553-71.

Sheiner, L. B. and S. L. Beal (1983). "Evaluation of methods for estimating population pharmacokinetic parameters. III. Monoexponential model: routine clinical pharmacokinetic data." J Pharmacokinet Biopharm **11**(3): 303-19.

Sheiner, L. B. and D. B. Rubin (1995). "Intention-to-treat analysis and the goals of clinical trials." Clin Pharmacol Ther **57**(1): 6-15.

Shimizu, M., T. Fukami, Y. Kobayashi, M. Takamiya, Y. Aoki, M. Nakajima and T. Yokoi (2012). "A novel polymorphic allele of human arylacetamide deacetylase leads to decreased enzyme activity." Drug Metab Dispos **40**(6): 1183-90.

Shmelev, N. A., L. N. Shabalova and V. P. Kolosovskaia (1975). "[Comparative clinical electroencephalographic study of cycloserine and terizidon tolerance]." Antibiotiki **20**(2): 174-80.

Sibbald, B. and M. Roland (1998). "Understanding controlled trials. Why are randomised controlled trials important?" Bmj **316**(7126): 201.

Siccardi, M., A. D'Avolio, L. Baietto, S. Gibbons, M. Sciandra, D. Colucci, S. Bonora, S. Khoo, D. J. Back, G. Di Perri and A. Owen (2008). "Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir." Clin Infect Dis **47**(9): 1222-5.

Siddiqi, K., M. L. Lambert and J. Walley (2003). "Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence." Lancet Infect Dis **3**(5): 288-96.

Sirgel, F. A., F. J. Botha, D. P. Parkin, B. W. Van De Wal, P. R. Donald, P. K. Clark and D. A. Mitchison (1993). "The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts: a new method of drug assessment." J Antimicrob Chemother **32**(6): 867-75.

Sirgel, F. A., P. R. Donald, J. Odhiambo, W. Githui, K. C. Umapathy, C. N. Paramasivan, C. M. Tam, K. M. Kam, C. W. Lam, K. M. Sole and D. A. Mitchison (2000). "A multicentre study of the early bactericidal activity of anti-tuberculosis drugs." J Antimicrob Chemother **45**(6): 859-70.

Sirgel, F. A., R. M. Warren, E. M. Streicher, T. C. Victor, P. D. van Helden and E. C. Bottger (2012). "gyrA mutations and phenotypic susceptibility levels to ofloxacin and moxifloxacin in clinical isolates of Mycobacterium tuberculosis." J Antimicrob Chemother **67**(5): 1088-93.

Smythe, W., A. Khandelwal, C. Merle, R. Rustomjee, M. Gninafon, M. Bocar Lo, O. B. Sow, P. L. Olliaro, C. Lienhardt, J. Horton, P. Smith, H. McIlleron and U. S. Simonsson (2012). "A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients." Antimicrob Agents Chemother **56**(4): 2091-8.

Somoskovi, A., C. Kodmon, A. Lantos, Z. Bartfai, L. Tamasi, J. Fuzy and P. Magyar (2000). "Comparison of recoveries of mycobacterium tuberculosis using the automated BACTEC

MGIT 960 system, the BACTEC 460 TB system, and Lowenstein-Jensen medium." J Clin Microbiol **38**(6): 2395-7.

Sonnenberg, P., J. Murray, J. R. Glynn, S. Shearer, B. Kambashi and P. Godfrey-Faussett (2001). "HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers." Lancet **358**(9294): 1687-93.

Sreevatsan, S., X. Pan, Y. Zhang, B. N. Kreiswirth and J. M. Musser (1997). "Mutations associated with pyrazinamide resistance in *pncA* of Mycobacterium tuberculosis complex organisms." Antimicrob Agents Chemother **41**(3): 636-40.

Sreevatsan, S., K. E. Stockbauer, X. Pan, B. N. Kreiswirth, S. L. Moghazeh, W. R. Jacobs, Jr., A. Telenti and J. M. Musser (1997). "Ethambutol resistance in Mycobacterium tuberculosis: critical role of *embB* mutations." Antimicrob Agents Chemother **41**(8): 1677-81.

Srivastava, S., S. Musuka, C. Sherman, C. Meek, R. Leff and T. Gumbo (2010). "Efflux-pump-derived multiple drug resistance to ethambutol monotherapy in Mycobacterium tuberculosis and the pharmacokinetics and pharmacodynamics of ethambutol." J Infect Dis **201**(8): 1225-31.

Stambaugh, J. J., S. E. Berning, A. E. Bulpitt, E. S. Hollender, M. Narita, D. Ashkin and C. A. Peloquin (2002). "Ofloxacin population pharmacokinetics in patients with tuberculosis." Int J Tuberc Lung Dis **6**(6): 503-9.

Steinberg, D. and M. Golovnya (2007). CART 6.0 Userguide. San Diego, California, Salford Systems.

Stottmeier, K. D. (1976). "Emergence of rifampin-resistant Mycobacterium tuberculosis in Massachusetts." J Infect Dis **133**(1): 88-90.

Straus, E. and N. Wu (1980). "Radioimmunoassay of tuberculo-protein derived from Mycobacterium tuberculosis." Proc Natl Acad Sci U S A **77**(7): 4301-4.

Suzuki, Y., C. Katsukawa, K. Inoue, Y. Yin, H. Tasaka, N. Ueba and M. Makino (1995). "Mutations in *rpoB* gene of rifampicin resistant clinical isolates of Mycobacterium tuberculosis in Japan." Kansenshogaku Zasshi **69**(4): 413-9.

Synold, T. W., I. Dussault and B. M. Forman (2001). "The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux." Nat Med **7**(5): 584-90.

Tappero, J. W., W. Z. Bradford, T. B. Agerton, P. Hopewell, A. L. Reingold, S. Lockman, A. Oyewo, E. A. Talbot, T. A. Kenyon, T. L. Moeti, H. J. Moffat and C. A. Peloquin (2005). "Serum concentrations of antimycobacterial drugs in patients with pulmonary tuberculosis in Botswana." Clin Infect Dis **41**(4): 461-9.

Telenti, A., P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer and T. Bodmer (1993). "Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis." Lancet **341**(8846): 647-50.



Thiebaut, F., T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan and M. C. Willingham (1987). "Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues." Proc Natl Acad Sci U S A **84**(21): 7735-8.

Tirona, R. G., B. F. Leake, G. Merino and R. B. Kim (2001). "Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans." J Biol Chem **276**(38): 35669-75.

Tirona, R. G., B. F. Leake, A. W. Wolkoff and R. B. Kim (2003). "Human organic anion transporting polypeptide-C (SLC21A6) is a major determinant of rifampin-mediated pregnane X receptor activation." J Pharmacol Exp Ther **304**(1): 223-8.

Tishkoff, S. A. and S. M. Williams (2002). "Genetic analysis of African populations: human evolution and complex disease." Nat Rev Genet **3**(8): 611-21.

Todar, K. (2012). The Growth of Bacterial Populations. Online Textbook of Bacteriology. Wisconsin, [http://textbookofbacteriology.net/growth\\_2.html](http://textbookofbacteriology.net/growth_2.html).

Tsukamura, M. (1972). "The pattern of resistance development to rifampicin in *Mycobacterium tuberculosis*." Tubercle **53**(2): 111-7.

van Zyl-Smit, R. N., A. Binder, R. Meldau, H. Mishra, P. L. Semple, G. Theron, J. Peter, A. Whitelaw, S. K. Sharma, R. Warren, E. D. Bateman and K. Dheda (2011). "Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden." PLoS One **6**(12): e28815.

Varughese, A., D. C. Brater, L. Z. Benet and C. S. Lee (1986). "Ethambutol kinetics in patients with impaired renal function." Am Rev Respir Dis **134**(1): 34-8.

Vassall, A., S. van Kampen, H. Sohn, J. S. Michael, K. R. John, S. den Boon, J. L. Davis, A. Whitelaw, M. P. Nicol, M. T. Gler, A. Khaliqov, C. Zamudio, M. D. Perkins, C. C. Boehme and F. Cobelens (2011). "Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis." PLoS Med **8**(11): e1001120.

Visser, M. E., H. M. Grewal, E. C. Swart, M. A. Dhansay, G. Walzl, S. Swanevelder, C. Lombard and G. Maartens (2010). "The effect of vitamin A and zinc supplementation on treatment outcomes in pulmonary tuberculosis: a randomized controlled trial." Am J Clin Nutr **93**(1): 93-100.

Visser, M. E., M. C. Stead, G. Walzl, R. Warren, M. Schomaker, H. M. Grewal, E. C. Swart and G. Maartens (2012). "Baseline predictors of sputum culture conversion in pulmonary tuberculosis: importance of cavities, smoking, time to detection and w-beijing genotype." PLoS One **7**(1): e29588.

Voogt, G. R. and H. S. Schoeman (1996). "Ototoxicity of aminoglycoside drugs in tuberculosis treatment." S Afr J Commun Disord **43**: 3-6.

Vuilleumier, N., M. F. Rossier, A. Chiappe, F. Degoumois, P. Dayer, B. Mermillod, L. Nicod, J. Desmeules and D. Hochstrasser (2006). "CYP2E1 genotype and isoniazid-induced hepatotoxicity in patients treated for latent tuberculosis." Eur J Clin Pharmacol **62**(6): 423-9.

Wang, X. D., X. Y. Deng, J. Chen, J. L. Li, X. Chen, L. Z. Zhao, Y. Lu, B. Chowbay, Q. B. Su, M. Huang and S. F. Zhou (2008). "Single nucleotide polymorphisms of the pregnane x receptor gene in Han Chinese and a comparison with other ethnic populations." Pharmacology **81**(4): 350-4.

Wayne, L. G. and L. G. Hayes (1996). "An in vitro model for sequential study of shift down of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence." Infect Immun **64**(6): 2062-9.

Weber, F. J. (1948). "Decline of tuberculosis mortality." Public Health Rep **63**(6): 161.

Weber, W. W. and D. W. Hein (1979). "Clinical pharmacokinetics of isoniazid." Clin Pharmacokinet **4**(6): 401-22.

Wehrli, W. (1983). "Rifampin: mechanisms of action and resistance." Rev Infect Dis **5** Suppl 3: S407-11.

Weiner, I. M. and J. P. Tinker (1972). "Pharmacology of pyrazinamide: metabolic and renal function studies related to the mechanism of drug-induced urate retention." J Pharmacol Exp Ther **180**(2): 411-34.

Weiner, M., D. Benator, W. Burman, C. A. Peloquin, A. Khan, A. Vernon, B. Jones, C. Silva-Trigo, Z. Zhao and T. Hodge (2005). "Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis." Clin Infect Dis **40**(10): 1481-91.

Weiner, M., W. Burman, A. Vernon, D. Benator, C. A. Peloquin, A. Khan, S. Weis, B. King, N. Shah and T. Hodge (2003). "Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine." Am J Respir Crit Care Med **167**(10): 1341-7.

Weiner, M., C. Peloquin, W. Burman, C. C. Luo, M. Engle, T. J. Prihoda, W. R. Mac Kenzie, E. Bliven-Sizemore, J. L. Johnson and A. Vernon (2010). "Effects of tuberculosis, race, and human gene *SLCO1B1* polymorphisms on rifampin concentrations." Antimicrob Agents Chemother **54**(10): 4192-200.

Weiner, M., T. J. Prihoda, W. Burman, J. L. Johnson, S. Goldberg, N. Padayatchi, P. Duran, M. Engle, G. Muzanye, R. D. Mugerwa and A. W. Sturm (2010). "Evaluation of time to detection of *Mycobacterium tuberculosis* in broth culture as a determinant for end points in treatment trials." J Clin Microbiol **48**(12): 4370-6.

WHO (2008). "Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. Geneva, Switzerland." 11.

WHO. (2008). "South African National Tuberculosis Management Guidelines." Retrieved 20 December 2012, from [http://www.who.int/hiv/pub/guidelines/south\\_africa\\_tb.pdf](http://www.who.int/hiv/pub/guidelines/south_africa_tb.pdf).

WHO (2012). Global Tuberculosis Report, World Health Organization: [http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf).

Wilkins, J. J., G. Langdon, H. McIlleron, G. Pillai, P. J. Smith and U. S. Simonsson (2011). "Variability in the population pharmacokinetics of isoniazid in South African tuberculosis patients." Br J Clin Pharmacol **72**(1): 51-62.

Wilkins, J. J., G. Langdon, H. McIlleron, G. C. Pillai, P. J. Smith and U. S. Simonsson (2006). "Variability in the population pharmacokinetics of pyrazinamide in South African tuberculosis patients." Eur J Clin Pharmacol **62**(9): 727-35.

Wilkins, J. J., R. M. Savic, M. O. Karlsson, G. Langdon, H. McIlleron, G. Pillai, P. J. Smith and U. S. Simonsson (2008). "Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption." Antimicrob Agents Chemother **52**(6): 2138-48.

Willyard, C. (2012). "Race heats up for first-to-market drugs for resistant tuberculosis." Nat Med **18**(8): 1157.

Woo, P. C., S. K. Lau, J. L. Teng, H. Tse and K. Y. Yuen (2008). "Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories." Clin Microbiol Infect **14**(10): 908-34.

Woo, P. C., J. L. Teng, J. M. Yeung, H. Tse, S. K. Lau and K. Y. Yuen (2011). "Automated identification of medically important bacteria by 16S rRNA gene sequencing using a novel comprehensive database, 16SpathDB." J Clin Microbiol **49**(5): 1799-809.

Wright, D. H., G. H. Brown, M. L. Peterson and J. C. Rotschafer (2000). "Application of fluoroquinolone pharmacodynamics." J Antimicrob Chemother **46**(5): 669-83.

Yajko, D. M., C. Wagner, V. J. Tevere, T. Kocagoz, W. K. Hadley and H. F. Chambers (1995). "Quantitative culture of Mycobacterium tuberculosis from clinical sputum specimens and dilution endpoint of its detection by the Amplicor PCR assay." J Clin Microbiol **33**(7): 1944-7.

Yeager, R. L., W. G. Munroe and F. I. Dessau (1952). "Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis." Am Rev Tuberc **65**(5): 523-46.

Yew, W. W., C. K. Chan, C. H. Chau, C. M. Tam, C. C. Leung, P. C. Wong and J. Lee (2000). "Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin/levofloxacin-containing regimens." Chest **117**(3): 744-51.

Zent, C. and P. Smith (1995). "Study of the effect of concomitant food on the bioavailability of rifampicin, isoniazid and pyrazinamide." Tuber Lung Dis **76**(2): 109-13.

Zhang, C., P. Denti, E. Decloedt, G. Maartens, M. O. Karlsson, U. S. Simonsson and H. McIlleron (2012). "Model-based approach to dose optimization of lopinavir/ritonavir when co-administered with rifampicin." Br J Clin Pharmacol **73**(5): 758-67.

Zhang, C., H. McIlleron, Y. Ren, J. S. van der Walt, M. O. Karlsson, U. S. Simonsson and P. Denti (2012). "Population pharmacokinetics of lopinavir and ritonavir in combination with rifampicin-based antitubercular treatment in HIV-infected children." Antivir Ther **17**(1): 25-33.

Zhang, J., P. Kuehl, E. D. Green, J. W. Touchman, P. B. Watkins, A. Daly, S. D. Hall, P. Maurel, M. Relling, C. Brimer, K. Yasuda, S. A. Wrighton, M. Hancock, R. B. Kim, S. Strom, K. Thummel, C. G. Russell, J. R. Hudson, Jr., E. G. Schuetz and M. S. Boguski (2001). "The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants." Pharmacogenetics **11**(7): 555-72.

Zhang, Y., M. M. Wade, A. Scorpio, H. Zhang and Z. Sun (2003). "Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid." J Antimicrob Chemother **52**(5): 790-5.

Zhang, Y., W. W. Yew and M. R. Barer (2012). "Targeting persisters for tuberculosis control." Antimicrob Agents Chemother **56**(5): 2223-30.

Zhu, M., W. J. Burman, J. R. Starke, J. J. Stambaugh, P. Steiner, A. E. Bulpitt, D. Ashkin, B. Auclair, S. E. Berning, R. W. Jelliffe, G. S. Jaresko and C. A. Peloquin (2004). "Pharmacokinetics of ethambutol in children and adults with tuberculosis." Int J Tuberc Lung Dis **8**(11): 1360-7.

Zignol, M., M. S. Hosseini, A. Wright, C. L. Weezenbeek, P. Nunn, C. J. Watt, B. G. Williams and C. Dye (2006). "Global incidence of multidrug-resistant tuberculosis." J Infect Dis **194**(4): 479-85.

Zorzoli, P., F. Moncalvo and G. C. Moreo (1962). "[On the antimycobacterial activity of rifamycin SV. Experimental research "in vitro" and "in vivo" and administration by local route in clinical practice]." G Ital Tuberc Mal Torace **16**: 226-34.

Zvada, S. P., P. Denti, H. Geldenhuys, S. Meredith, D. van As, M. Hatherill, W. Hanekom, L. Wiesner, U. S. Simonsson, A. Jindani, T. Harrison and H. M. McIlleron (2012). "Moxifloxacin population pharmacokinetics in patients with pulmonary tuberculosis and the effect of intermittent high-dose rifapentine." Antimicrob Agents Chemother **56**(8): 4471-3.

Zvada, S. P., J. S. Van Der Walt, P. J. Smith, P. B. Fourie, G. Roscigno, D. Mitchison, U. S. Simonsson and H. M. McIlleron (2010). "Effects of four different meal types on the population pharmacokinetics of single-dose rifapentine in healthy male volunteers." Antimicrob Agents Chemother **54**(8): 3390-4.

Zweig, M. H. and G. Campbell (1993). "Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine." Clin Chem **39**(4): 561-77.

Zwietering, M. H., I. Jongenburger, F. M. Rombouts and K. van 't Riet (1990). "Modeling of the bacterial growth curve." Appl Environ Microbiol **56**(6): 1875-81.

## APPENDICES

### Appendix 1 – Ethical approval for DELFT study

UNIVERSITY OF CAPE TOWN



Research Ethics Committee  
E52 Room 24, Old Main Building Groote  
Schoor Hospital, Observatory, 7925  
Queries : Lamees Emjedi  
Tel : (021) 406-6338 Fax: 406-6411  
E-mail : lemjedi@curie.uct.ac.za

01 July 2005

**REC REF: 194/2005**

Dr. H MCIlleron  
Pharmacology

Dear MCIlleron

**THE EFFECT OF THE PHARMACOKINETICS OF RIFAMPICIN, ISONIAZID,  
PYRAZINAMIDE & ETHAMBUTOL ON THE BACTERIOLOGIC RESPONSE  
OF PERSONS WITH PULMONARY TUBERCULOSIS IN THE WESTERN  
CAPE**

Thank you for your letter to the Research Ethics Committee dated  
21/06/2005.

It is a pleasure to inform you that the Ethics Committee has formally approved  
the above mentioned study.

**Please quote the REC. REF in all your correspondence.**

Yours sincerely

**PROF. T. ZABOW**  
**CHAIRPERSON**

UNIVERSITY OF CAPE TOWN



Health Sciences Faculty  
Research Ethics Committee  
Room E52-24 Groote Schuur Hospital Old Main Building  
Observatory 7925  
Telephone [021] 406 6338 • Facsimile [021] 406 6411  
e-mail: sumayah.ariiefdien@uct.ac.za

04 November 2009

REC REF: 194/2005

Dr H McIlleron  
Clinical Pharmacology  
K45  
Old Main Building

Dear Dr McIlleron

PROJECT TITLE: THE EFFECT OF THE PHARMACOKINETICS OF RIFAMPICIN, ISONIAZID, PYRAZINAMIDE AND ETHAMBUTOL ON THE BACTERIOLOGIC RESPONSE OF PERSONS WITH PULMONARY TUBERCULOSIS IN THE WESTERN CAPE.

RE: GENOTYPING DELFT STUDY PATIENTS

Thank you for your letter to the Research Ethics Committee dated 29 October 2009.

The genetic test proposed for the above study is covered by the original IRB approval (REC REF 194/2005)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

**PROFESSOR M. BLOCKMAN**  
**CHAIRPERSON, HSF HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.  
Institutional Review Board (IRB) number: IRB00001938

lemjedi

## Appendix 2 – Ethical approval for MDR-TB study in Cape Town



UNIVERSITY OF CAPE TOWN

Health Sciences Faculty  
Research Ethics Committee  
Room E52-24 Groote Schuur Hospital Old Main Building  
Observatory 7925  
Telephone [021] 406 6338 • Facsimile [021] 406 6411  
e-mail: lamees.emjedi@uct.ac.za

28 April 2009

REC REF: 152/2009

**Dr H McIlleron**  
Clinical Pharmacology  
K45  
Old Main Building

Dear Dr H McIlleron

**PROJECT TITLE: POPULATION PHARMACOKINETICS OF SECOND LINE ANTI-TUBERCULOSIS DRUGS FOR MULTI-DRUG RESISTANT TUBERCULOSIS**

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

**Approval is granted for one year till the 30<sup>th</sup> April 2010.**

Please send us an annual progress report if your research continues beyond the approval period. Alternatively, please send us a brief summary of your findings so that we can close the research file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the REC. REF in all your correspondence.**

Yours sincerely

pp

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, HSF HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.

lemjedi



Verwysing  
Reference  
Iselathiso

19/18/RP 09/2010

Navrae  
Enquiries  
Imibuzo

Dr N Peer

Telefoon  
Telephone  
Ifowuni

021 483 6858

K-45 Old Main Building  
Groote Schuur Hospital  
Observatory  
7925  
FAX: 021 448 1989

Departament van Gesondheid  
Department of Health  
ISebe lezeMpilo

Dear Mr E Chigutsa

**RE: POPULATION PHARMACOKINETICS OF SECOND LINE ANTI-TUBERCULOSIS DRUGS FOR MULTI-DRUG RESISTANT TUBERCULOSIS**

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research. Please contact the following members of staff to assist you with access to the facility:

DP Marais: Dr J Mobbs Email: [Johmobbs@pgwc.gov.za](mailto:Johmobbs@pgwc.gov.za) Tel No.: 021-7137507

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator ([healthres@pgwc.gov.za](mailto:healthres@pgwc.gov.za)).
3. The reference number above should be quoted in all future correspondence.

We look forward to hearing from you.

Yours sincerely

DR J CUPIDO  
DEPUTY-DIRECTOR GENERAL  
DISTRICT HEALTH SERVICES AND PROGRAMMES  
DATE:

CC: DR J MOBBS

CEO DP Marais Hospital

Page 1 of 2

Dorpstraat 4  
Postbus 2060  
KAAPSTAD  
8000

4 Dorp Street  
PO Box 2060  
CAPE TOWN  
8000



## Appendix 3 – NONMEM CONTROL FILES

### 3.1 Rifampicin Control stream

```
$PROBLEM Rifampicin final model
$INPUT ID DMID=DROP OCC TIME LDV=DROP DV AMT MDV EVID WT SEX HT
HIV AGE BQL SLCO
$DATA RIF_COMPLETE.csv IGNORE=#
$ABBREVIATED COMRES=1 ; FOR CMAX AND TMAX DETERMINATION
$SUBROUTINES ADVAN6 TOL=6
```

```
$MODEL
NCOMPARTMENTS=5
COMP=(ABS DEFDOSE) ; Actually the dose won't go there. F1=0
COMP=(CENTRAL DEFOBSERVATION) ; CENTRAL COMPARTMENT
COMP=(TTMAX) ; FOR WORKING OUT TMAX
COMP=(CCMAX) ; FOR WORKING OUT CMAX
COMP=(AUC) ; FOR WORKING OUT AUC
```

```
$PK
```

```
"FIRST
" COMMON/PRCOMG/ IDUM1,IDUM2,IMAX,IDUM4,IDUM5
"INTEGER IDUM1,IDUM2,IMAX,IDUM4,IDUM5
"IMAX=10000000
```

```
;-----CALCULATE TIME AFTER DOSE -----
IF(AMT.GT.0)TDOS = TIME
TAD=TIME-TDOS
;----- END TAD CALCULATION -----
```

```
;-----DEFINE RANDOM EFFECTS-----
```

```
BSVCL=ETA(1) ; BSV FOR CL
BSVMT=ETA(2) ; BSV FOR MTT
```

```
IF(OCC.EQ.1) THEN
WSVCL=ETA(3) ; WSV FOR CL OCC=1
WSVMT=ETA(5) ; WSV FOR MTT OCC=1
WSVV=ETA(6) ; WSV FOR V OCC=1
ENDIF
```

```
IF(OCC.EQ.2) THEN
WSVCL=ETA(4) ; WSV FOR CL OCC=2
WSVMT=ETA(7) ; WSV FOR MTT OCC=2
WSVV=ETA(8) ; WSV FOR V OCC=2
ENDIF
```

```
;-----END DEFINE RANDOM EFFECTS-----
```

```
TVCL=THETA(1)*((WT/70)**0.75)
CL=TVCL*EXP(BSVCL+WSVCL) ; CLEARANCE
```

```
TVV2=THETA(2)*(WT/70)*(1-THETA(3)*SEX)
V2=TVV2*EXP(WSVV) ; VOLUME
```

```
KA=THETA(4)
```

```

        IF(SLCO.EQ.0) MUT=0          ; WILD TYPE INDIVIDUAL. NO rs4149032
POLYMORPHISM
        IF(SLCO.EQ.1) MUT=THETA(5)    ; HETEROZYGOUS INDIVIDUAL FOR rs4149032
POLYMORPHISM
        IF(SLCO.EQ.2) MUT=THETA(6)    ; HOMOZYGOUS INDIVIDUAL FOR rs4149032
POLYMORPHISM

        IF(SLCO.EQ.3) THEN            ; MIXTURE MODEL FOR INDIVIDUALS WITH NO
GENOTYPE DATA
        IF(MIXNUM.EQ.1) THEN
            MUT=0                      ; WILD TYPE INDIVIDUALS
        ELSE
            IF(MIXNUM.EQ.2) THEN
                MUT=THETA(6)           ; EITHER A HETEROZYGOTE OR HOMOZYGOTE, BUT
TREATED AS HOMOZYGOTE
            ENDIF
        ENDIF
    ENDIF

    BIO=THETA(7)*(1-MUT)               ; BIOAVAILABILITY. THETA(2) FIXED TO 1

    DEF=PODO/150                      ; CONTINUOUS EFFECT OF DOSE ON MTT DIVIDED BY
150MG TABLET STRENGTH
    TVMTT=THETA(8)*(1+THETA(3)*SEX)*(1-THETA(9)*(DEF-3))
    MTT = TVMTT*EXP(BSVMT+WSVMT)      ; MEAN TRANSIT TIME

    NN = THETA(10)                   ; NUMBER OF TRANSIT COMPARTMENTS

;-----CODE FOR TRANSIT ABSORPTION COMPARTMENT MODEL-----
    KTR = (NN+1)/MTT                 ; TRANSIT COMPT RATE CONSTANT
    L = 0.9189385+(NN+.5)*LOG(NN)-NN+LOG(1+1/(12*NN)) ; STIRLING'S APPROXIMATION
FOR TRANSIT COMPARTMENT MODEL
    F1 = 0                          ; SO THAT DOSE ENTERS VIA TRANSIT COMPARTMENTS
    IF(AMT.GT.0)PODO = AMT
    X = 1E-6                        ; TO AVOID LOG OF ZERO
    LPOD=LOG(PODO*BIO+X)             ; BIOAVAILABILITY IS INCORPORATED HERE
    LKTR= LOG(KTR+X)
    LPKT=LPOD+LKTR - L
;-----END FOR TRANSIT COMPARTMENT MODEL-----

$MIX
NSPOP=2                            ; MIXTURE MODEL FOR THOSE WHO DID NOT HAVE GENOTYPE DATA
P(1)=THETA(11)
P(2)=1-P(1)

    S2 = V2
    K = CL/V2

$DES
DEL = 1E-6                          ; TO PREVENT LOG OF ZERO ERROR

IF(T.GE.TDOS)THEN
    DADT(1)=EXP(LPKT+NN*LOG(KTR*(T-TDOS)+DEL)-KTR*(T-TDOS))-KA*A(1) ; TRANSIT
ABSORPTION MODEL
ELSE
    DADT(1)=0
ENDIF
DADT(2)=KA*A(1)-K*A(2)              ; CENTRAL COMPARTMENT

```

```

XXX=KA*A(1)-K*A(2)          ; TO WORK OUT CMAX BELOW. NEED DERIVATIVE OF
CENTRAL COMPT

COM(1)=0
IF (TIME.GT.72) THEN      ; PK SAMPLING DOSE WAS GIVEN AT 72H FOR ALL PATIENTS IN THE
DATASET.BUT 3 PREVIOUS DOSES RECORDED
  IF(XXX.GT.0) COM(1)=1 ; A2 INCREASING
ENDIF

IF (TIME.GT.72) THEN
  IF(XXX.EQ.0) COM(1)=2 ; A2 MAXIMUM
ENDIF

IF (TIME.GT.72) THEN
  IF(XXX.LT.0) COM(1)=3 ; A2 DECREASING
ENDIF

IF(COM(1).LT.3)THEN      ; TIME TO CMAX. TMAX
DADT(3) = 1
ELSE
DADT(3) = 0
ENDIF

IF(COM(1).LT.3)THEN      ; MAXIMUM A2
DADT(4) = XXX
ELSE
DADT(4) = 0
ENDIF

IF (TIME.LE.72) THEN
DADT(5)=0      ; DO NOT INTEGRATE AMOUNTS FOR THE 3 PREVIOUS DOSES (0, 24, 48
H) TO CALCULATE AUC
ELSE
DADT(5)=A(2)/V2
ENDIF

$ERROR
IPRED=F
  ADD=THETA(12)
  PROP=IPRED*THETA(13)
  W=SQRT(ADD*ADD + PROP*PROP)
  IF(W.LE.0) W=1
  IRES=DV-IPRED
  IWRES=IRES/W

;-----M3 METHOD FOR BQL DATA-----

SIG  = W
LOQ  = 0.08      ; LLOQ
DUM  = (LOQ - IPRED) / SIG
CUMD = PHI(DUM)
IF (BQL.EQ.0) THEN      ; TREAT DV AS AN OBSERVATION
F_FLAG = 0
Y      = IPRED + SIG * ERR(1)
ENDIF
IF (BQL.EQ.1) THEN      ; TREAT DV AS A LIKELIHOOD
F_FLAG = 1
Y      = CUMD
ENDIF

```

TMAX = A(3)  
 CMAX = A(4)/V2 ; AMOUNT DIVIDED BY THE CENTRAL VOLUME OF DISTRIBUTION =>  
 CMAX  
 RAUC=A(5)

STRT=0  
 IF(MIXNUM.EQ.1) STRT=0 ; OUTPUT SPECIFIC SUBPOPULATION FOR PATIENTS WITH  
 MIXTURE MODEL. WILD TYPE  
 IF(MIXNUM.EQ.2) STRT=2 ; OUTPUT SPECIFIC SUBPOPULATION FOR PATIENTS WITH  
 MIXTURE MODEL. HOMOZYGOTES

\$THETA  
 (0,10.6) ; CLEARANCE  
 (1,46.1) ; VOLUME  
 (0,0.273,0.5) ; SEX on MTT and V  
 (0,0.98) ; KA  
 (0,0.219,0.5) ; FRACTIONAL EFFECT OF SLCO ON BIOAVAILABILITY IN HETEROZYGOTES  
 (0,0.282,0.7) ; FRACTIONAL EFFECT OF SLCO ON BIOAVAILABILITY IN HOMOZYGOTES  
 1 FIX ; BIOAVAILABILITY FOR WILD TYPE  
 (0,1.5) ; MEAN TRANSIT TIME (MTT)  
 (-0.5,0.281,1) ; FRACTIONAL EFFECT OF DOSE ON MTT  
 (1,19) FIX ; NN FIXED TO VALUE FROM PREVIOUS MODEL IN ORDER TO TRY TO GET  
 COVARIANCE STEP TO RUN  
 (0,0.0719) ; PROPORTION WITH F1=1  
 (0,0.0342) ; ADDITIVE ERROR  
 (0,0.298) ; PROPORTIONAL ERROR

\$OMEGA BLOCK(2)  
 0.0541 ; BSV\_CL  
 0.157 0.499 ; BSV\_MTT  
 \$OMEGA BLOCK(1) 0.18 ; WSV\_CL OCC=1  
 \$OMEGA BLOCK(1) SAME ; WSV\_CL OCC=2  
 \$OMEGA BLOCK(2)  
 0.359 ; WSV\_MTT OCC=1  
 -0.0756 0.0901 ; WSV\_V OCC=1  
 \$OMEGA BLOCK(2) SAME ; WSV\_MTT OCC=2 WSV\_V OCC=2

\$SIGMA 1 FIX  
 \$ESTIMATION MAXEVAL=9990 PRINT=3 SIGDIG=3 NOABORT MSFO=MSF1a  
 METHOD=1 LAPLACIAN INTERACTION

\$TABLE ID TIME OCC DV IPRED RES IRES IWRES SLCO DEF NOPRINT ONEHEADER  
 FILE=SDTAB1a  
 \$TABLE ID TIME CL KA V2 BIO OCC MTT STRT BSVB BSVCL BSVV BSVMT BSVKA BOVCL  
 WSVV WSVMT RAUC TMAX CMAX NOPRINT ONEHEADER FILE=PATAB1a  
 \$TABLE ID WT HT AGE NOPRINT ONEHEADER FILE=COTAB1a  
 \$TABLE ID SEX HIV SLCO OCC NOPRINT ONEHEADER FILE=CATAB1a

### 3.2 Pyrazinamide control stream

```
$PROBLEM Pyrazinamide final model
$INPUT ID DMID=DROP OCC WHAT=DROP TIME DRUG=DROP CONC=DROP DV BQL AMT
SS=DROP
II=DROP MDV EVID BLND SEX AGE HT WT BSA BMI HIV MUAC TSF BSF SSDX

$DATA PZA.csv IGNORE=#
$DATA IGNORE (BQL.EQ.1) ; IGNORE THE 2 BQL POINTS

$SUBROUTINES ADVAN6 TOL=3
$MODEL NCOMP=2
      COMP=(DEPOT, DEFDOSE) ; DOSING COMPARTMENT
      COMP=(CENTRAL, DEFOBS) ; CENTRAL COMPARTMENT

$PK

"FIRST
"      COMMON/PRCOMG/ IDUM1,IDUM2,IMAX,IDUM4,IDUM5
"INTEGER IDUM1,IDUM2,IMAX,IDUM4,IDUM5
"IMAX=10000000

;-----FOR WSV-----
OC1=0
OC2=0
IF(OCC.EQ.1) OC1=1
IF(OCC.EQ.2) OC2=1
;-----END FOR WSV-----

IF(AMT.GT.0)PODO = AMT

;-----CALCULATE TIME AFTER DOSE -----
IF(AMT.GT.0)TDOS = TIME
TAD=TIME-TDOS
;----- END TAD CALCULATION -----

TDX=THETA(1)*EXP(OC1*ETA(1) + OC2*ETA(2) + ETA(3)) ; TDX IS TIME OF CHANGING FROM
LOW KA TO HIGH KA

MTDIFF=1

;-----THE CODE BELOW IS NECESSARY BECAUSE THE DATASET HAS 3 DOSES BEFORE
THE DOSE TAKEN WITH THE PK SAMPLING.
;----- SO IT IS NECESSARY TO BE ABLE TO HAVE TIME DEPENDENT KA FOR EACH OF
THOSE DOSES-----
;-----BECAUSE ONCE MPAST BECOMES 1, IT WILL NEVER CHANGE BACK TO ZERO-----
-----

IF(NEWIND.NE.2) THEN
      COUNT=0 ; INITIALIZATION
      FLAG1=0
      FLAG2=0
      FLAG3=0
      FLAG4=0
      T1=1000
      T2=1000
      T3=1000
      T4=1000
      NEWOCC=0
ENDIF
```

```

IF (NEWOCC.EQ.1) THEN
    COUNT=0 ; INITIALIZATION
    FLAG1=0
    FLAG2=0
    FLAG3=0
    FLAG4=0
    T1=1000
    T2=1000
    T3=1000
    T4=1000
    NEWOCC=2
ENDIF

IF (AMT.GT.0.AND.COUNT.EQ.3) THEN
    COUNT=4
    T4=TIME+TDX
    FLAG3=0
    FLAG4=1
ENDIF

IF (AMT.GT.0.AND.COUNT.EQ.2) THEN
    COUNT=3
    T3=TIME+TDX
    FLAG2=0
    FLAG3=1
ENDIF

IF (AMT.GT.0.AND.COUNT.EQ.1) THEN
    COUNT=2
    T2=TIME+TDX
    FLAG1=0
    FLAG2=1
ENDIF

IF (AMT.GT.0.AND.COUNT.EQ.0) THEN
    COUNT=1
    T1=TIME+TDX
    FLAG1=1
    CIAO1=1
ENDIF

    MTIME(4)=T4
    MTIME(3)=T3
    MTIME(2)=T2
    MTIME(1)=T1

    TKA1=THETA(2) ; LOW KA
    TKA2=THETA(3)*EXP(OC1*ETA(4) + OC2*ETA(5)) ; HIGH KA, WHICH HAS WSV

    KA1=FLAG1*(TKA1*(1-MPAST(1)) + TKA2*MPAST(1)) ; FOR DOSE TAKEN 3 DAYS AGO
    KA2=FLAG2*(TKA1*(1-MPAST(2)) + TKA2*MPAST(2)) ; FOR DOSE TAKEN 2 DAYS AGO
    KA3=FLAG3*(TKA1*(1-MPAST(3)) + TKA2*MPAST(3)) ; FOR DOSE TAKEN 1 DAYS AGO
    KA4=FLAG4*(TKA1*(1-MPAST(4)) + TKA2*MPAST(4)) ; FOR DOSE TAKEN ON PK
SAMPLING DAY

    KA=KA1+KA2+KA3+KA4 ; TIME DEPENDENT, SEQUENTIAL, DUAL KA

    VM=THETA(4)*((WT/70)**0.75) ; VMAX

```

```

      KM=THETA(5) ; KM
      CL=THETA(6)*((WT/70)**0.75) ; FIRST ORDER CL
      BSVCL2=ETA(6) ; BSV IN COMBINED CL
      WSVCL2=OC1*ETA(7) + OC2*ETA(8)
      VARCL2=EXP(BSVCL2+WSVCL2) ; TOTAL VARIABILITY IN THE ELIMINATION OF
PYRAZINAMIDE

      V2=THETA(7)*WT/70 ; VOLUME

      S2=V2

      CLM=1+THETA(8)*SEX ; COVARIATE FOR MALE SEX ON BIOAVAILABILITY

      F1=THETA(9)*CLM*EXP(ETA(9)) ; BIOAVAILABILITY

$DES
C2=A(2)/S2 ; DRUG CONCENTRATION IN CENTRAL COMPARTMENT
CL2=(CL+VM/(C2+KM))*VARCL2 ; COMBINED MIXED ORDER AND FIRST ORDER
ELIMINATION
DADT(1)= -KA*A(1) ; DOSING COMPARTMENT
DADT(2)=KA*A(1)-CL2*A(2)/V2 ; CENTRAL COMPARTMENT

$ERROR
;-----for log transformed dv -----
IF (F.LE.0.000001) THEN
      IPRED= -13.816 ; IPRED is always very very small, unless the prediction (F) becomes
significant
ELSE
      IPRED=LOG(F)
ENDIF
;-----end for log transformed dv -----

IF(TAD.LT.1.5) THEN
      W=THETA(10) ; early proportional error
ELSE
      W=THETA(11) ; late proportional error
ENDIF
IF(W.EQ.0) W=1
IRES=DV-IPRED
IWRES=IRES/W
Y = IPRED + W*ERR(1)
IF(OCC.EQ.2.AND.NEWOCC.EQ.0) NEWOCC=1 ; TO CATER FOR TIME DEPENDENT KA IN
PATIENTS WITH 2 OCCASIONS

$THETA
(0,0.711237) ; MTIME. TIME OF CHANGING FROM LOW TO HIGH KA
(0,0.0212046) ; EARLY KA (LOW)
(0,0.989797) ; LATE KA (HIGH)
(0,14.3207) ; VMAX
(0,0.516623) ; KM
(0,2.63969) ; FIRST ORDER CL
(0,41.5993) ; VOLUME
-0.255895 ; MALE SEX ON BIO
1 FIX ; BIOAVAILABILITY
(0,0.42326) ; PROP ERR EARLY
(0,0.135336) ; PROP ERR LATE

$OMEGA BLOCK(1) 0.20422 ; BSV_MTIME
$OMEGA BLOCK(1) 0.230227 ; WSV_MTIME OCC=1

```

\$OMEGA BLOCK(1) SAME ; WSV\_MTIME OCC=2  
\$OMEGA BLOCK(1) 0.665198 ; WSV KA OCC=1  
\$OMEGA BLOCK(1) SAME ; WSV KA OCC=2  
\$OMEGA BLOCK(1) 0.0302231 ; BSV\_CL2  
\$OMEGA BLOCK(1) 0.0246831 ; WSV\_CL2 OCC=1  
\$OMEGA BLOCK(1) SAME ; WSV\_CL2 OCC=2  
\$OMEGA BLOCK(1) 0.0241555 ; PPV BIOAVAILABILITY

\$SIGMA 1 FIX

\$ESTIMATION MAXEVAL=9990 SIGDIG=3 PRINT=5 NOABORT MSFO=MSF7304b  
METHOD CONDITIONAL

\$TABLE ID TIME DV IPRED RES IRES IWRES OCC NOPRINT ONEHEADER FILE=SDTAB7304b  
\$TABLE ID TIME CL VM KM KA V2 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9  
NOPRINT ONEHEADER FILE=PATAB7304b  
\$TABLE ID WT HT AGE BSA NOPRINT ONEHEADER FILE=COTAB7304b  
\$TABLE ID SEX HIV BLND NOPRINT ONEHEADER FILE=CATAB7304b

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### 3.3 Isoniazid control stream

```
$PROBLEM Isoniazid final model
$DATA DELFT_2009-11-12.csv IGNORE=#
IGNORE=(DRUG.EQ.2,DRUG.EQ.3,DRUG.EQ.4) ; IGNORE OTHER DRUGS WHICH ARE NOT
ISONIAZID
$INPUT ID DMID=DROP OCC WHAT=DROP DAT1=DROP TIME DRUG RDV DV BQL AMT
SS=DROP II=DROP MDV EVID NTAB=DROP SEXM AGE HT WT BMI BSA HIV MUAC TSF BSF
SSDX L2=DROP
$ABBREVIATED COMRES=1 ; FOR CMAX AND TMAX DETERMINATION
$SUBROUTINE ADVAN6 TOL=3
$MODEL
NCOMPARTMENTS=5
COMP=(ABS DEFDOSE) ; Actually the dose won't go there. F1=0
COMP=(CENTRAL DEFOBSERVATION) ; CENTRAL COMPARTMENT
COMP=(TTMAX) ; FOR WORKING OUT TMAX
COMP=(CCMAX) ; FOR WORKING OUT CMAX
COMP=(AUC) ; FOR WORKING OUT AUC

$PK

;-----CALCULATE TIME AFTER DOSE -----
IF(AMT.GT.0)TDOS = TIME
TAD=TIME-TDOS
;----- END TAD CALCULATION -----

;-----FOR WSV-----
OC1=0
OC2=0
IF(OCC.EQ.1) OC1=1
IF(OCC.EQ.2) OC2=1
;-----END FOR WSV-----

IF(MIXNUM.EQ.1) THEN
TVCL=THETA(1)*(WT/70)**0.75 ; CL FOR FAST ACETYLATORS
ELSE
TVCL=THETA(2)*(WT/70)**0.75 ; CL FOR SLOW ACETYLATORS
ENDIF

CL=TVCL*EXP(ETA(1) + ETA(3)*OC1 + ETA(4)*OC2)

TVV2=THETA(3)*WT/70
V2=TVV2*EXP(ETA(2) + ETA(5)*OC1 + ETA(6)*OC2) ; VOLUME

TVKA=THETA(4)
KA=TVKA*EXP(ETA(1)) ; KA

TVMTT = THETA(5)
MTT = TVMTT*EXP(ETA(7)*OC1 + ETA(8)*OC2) ; MTT

;-----CODE FOR TRANSIT ABSORPTION COMPARTMENT MODEL-----

F1=0 ; dose must enter via transit compartments
BIO=1 ; bioavailability fixed to 1

IF(AMT.GT.0) PD=AMT

NN=THETA(6) ; number of transit compartments
KTR = (NN+1)/MTT
```

$L = 0.9189385 + (NN + 0.5) * \log(NN) - NN + \log(1 + 1/(12 * NN))$  ; logarithm of the gamma function  
 LBPDP=LOG(BIO\*PD)  
 LKTR=LOG(KTR)

PIZZA=LBPDP+LKTR-L

;-----END FOR TRANSIT COMPARTMENT MODEL-----

K20=CL/V2

S2=V2

\$MIX

NSPOP=2 ; 2 POPULATIONS (SLOW AND FAST ACETYLATORS)

P(1)=THETA(7) ; PROBABILITY OF BEING A FAST ACETYLATOR

P(2)=1-P(1) ; PROBABILITY OF NOT BEING A FAST ACETYLATOR (THEREFORE SLOW)

\$DES

TEMPO=T-TDOS

IF(TEMPO.GT.0)THEN

    KTT=KTR\*TEMPO

    DADT(1)=EXP(PIZZA+NN\*LOG(KTT)-KTT)-KA\*A(1) ; TRANSIT ABSORPTION MODEL

ELSE

    DADT(1)=0

ENDIF

DADT(2)=KA\*A(1)-K20\*A(2) ; CENTRAL COMPARTMENT

XXX=KA\*A(1)-K20\*A(2) ; TO WORK OUT CMAX BELOW. NEED DERIVATIVE OF CENTRAL COMPT

COM(1)=0

IF (TIME.GT.72) THEN ; OBSERVED DOSE WAS GIVEN AT 72H FOR ALL PATIENTS IN THE DATASET.

    IF(XXX.GT.0) COM(1)=1 ; A2 increasing

ENDIF

IF (TIME.GT.72) THEN

    IF(XXX.EQ.0) COM(1)=2 ; A2 maximum

ENDIF

IF (TIME.GT.72) THEN

    IF(XXX.LT.0) COM(1)=3 ; A2 decreasing

ENDIF

IF(COM(1).LT.3)THEN ; Time to Cmax

    DADT(3) = 1

ELSE

    DADT(3) = 0

ENDIF

IF(COM(1).LT.3)THEN ; Maximum A2

    DADT(4) = XXX

ELSE

    DADT(4) = 0

ENDIF

IF (TIME.LE.72) THEN

    DADT(5)=0 ; DO NOT INCLUDE AMOUNTS FOR THE 3 PREVIOUS DOSES (0, 24, 48 H) TO CALCULATE AUC

ELSE

    DADT(5)=A(2)/V2

```

ENDIF

$ERROR
;-----for log transformed dv -----
  IF (F.LE.0.000001) THEN
    IPRED= -13.816 ; IPRED is always very very small, unless the prediction (F) becomes
significant
  ELSE
    IPRED=LOG(F)
  ENDIF
;-----end for log transformed dv -----

  ADD=THETA(6)
  W = SQRT(ADD*ADD)
  IRES=DV-IPRED
  IWRES=IRES/W

;-----BEAL M3 METHOD-----
SIG  = W
LOQ  = 0.1 ; LLOQ
DUM  = (LOQ - IPRED) / SIG
CUMD = PHI(DUM)

IF (BQL.EQ.1) THEN ; treat DV as a likelihood for BQL data
  F_FLAG = 1
  Y      = CUMD
ELSE
  F_FLAG = 0
  Y      = IPRED + SIG * ERR(1) ; usual method for data that is above LLOQ
ENDIF

TMAX = A(3) ; TMAX
CMAX = A(4)/V2 ; Maximum amount divided by the central volume of distribution => Cmax
RAUC=A(5) ; AUC

$THETA
(0,24.9) ; CL FAST ACETYLATORS
(0,13) ; CL SLOW ACETYLATORS
(0,126) ; VOLUME
(0,3.58) ; KA
(0,0.693) ; MTT
(1,10) FIX ; NUMBER OF TRANSIT COMPARTMENTS
(0,0.544,1) ; PROPORTION WITH HIGH CL (FAST ACETYLATORS)
(0,0.235) ; PROPORTIONAL ERROR

$OMEGA BLOCK(2)
  0.285 ; BSV CL
  0.314 0.571 ; COVARIANCE BSV V
$OMEGA BLOCK(1) 0.0921 ; WSV CL OCC=1
$OMEGA BLOCK(1) SAME ; WSV CL OCC=2
$OMEGA BLOCK(1) 0.119 ; WSV V OCC=1
$OMEGA BLOCK(1) SAME ; WSV V OCC=2
$OMEGA BLOCK(1) 1.45 ; WSV_MTT OCC=1
$OMEGA BLOCK(1) SAME ; WSV_MTT OCC=2

$SIGMA 1 FIX

$ESTIMATION MAXEVAL=9990 SIGDIG=3 PRINT=5 NOABORT MSFO=MSF11b
METHOD=1 LAPLACIAN

```

\$COV UNCONDITIONAL MATRIX S

\$TABLE ID OCC TIME TAD CL KA V2 F1 DV HIV SEXM Y IPRED RAUC TMAX CMAX PD  
NOAPPEND NOPRINT ONEHEADER FILE=MYTAB11b

\$TABLE ID TIME TAD DV IPRED RES IRES IWRES OCC NOPRINT ONEHEADER  
FILE=SDTAB11b

\$TABLE ID TIME TAD CL KA V2 F1 MTT ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8  
NOPRINT ONEHEADER FILE=PATAB11b

\$TABLE ID WT HT MUAC TSF BSF AGE BSA NOPRINT ONEHEADER FILE=COTAB11b

\$TABLE ID SEXM HIV NOPRINT ONEHEADER FILE=CATAB11b

\$SCATTER DV VS PRED UNIT

\$SCATTER DV VS IPRED UNIT

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### 3.4 Ethambutol control stream

```
$PROBLEM Ethambutol final model
$DATA DELFT_2009-11-12.csv IGNORE=#
IGNORE=(DRUG.EQ.1,DRUG.EQ.2,DRUG.EQ.3) ; IGNORE OTHER DRUGS WHICH ARE NOT
ETHAMBUTOL
$INPUT ID DMID=DROP OCC WHAT=DROP DAT1=DROP TIME DRUG DV LDV BLQ AMT
SS=DROP II=DROP MDV EVID NTAB=DROP SEXM AGE HT WT BMI BSA HIV MUAC TSF BSF
SSDX

$SUBROUTINE ADVAN13 TOL=8
$MODEL
NCOMPARTMENTS=2
COMP=(ABS DEFDOSE) ; Actually the dose won't go there. F1=0
COMP=(CENTRAL DEFOBSERVATION) ; CENTRAL COMPARTMENT

$PK

;-----CALCULATE TIME AFTER DOSE -----
IF(AMT.GT.0)TDOS = TIME
TAD=TIME-TDOS
;----- END TAD CALCULATION -----

;-----FOR WSV-----
OC1=0
OC2=0
IF(OCC.EQ.1) OC1=1
IF(OCC.EQ.2) OC2=1
;-----END FOR WSV-----

CL=(THETA(1)*(WT/70)**0.75)*EXP(ETA(1) + ETA(2)*OC1 + ETA(3)*OC2) ; CLEARANCE

TVV2=THETA(2)*WT/70
V2=TVV2*EXP(ETA(4)*OC1 + ETA(5)*OC2) ; VOLUME

TVKA=THETA(3)
KA=TVKA ; KA

TVMTT = THETA(4)
MTT = TVMTT*EXP(ETA(6)*OC1 + ETA(7)*OC2)

;-----CODE FOR TRANSIT ABSORPTION COMPARTMENT MODEL-----
F1=0
BIO=1 ; bioavailability fixed to 1
IF(AMT.GT.0) PD=AMT

NN=THETA(5) ; number of transit compartments
KTR = (NN+1)/MTT
L = 0.9189385+(NN+.5)*LOG(NN)-NN+LOG(1+1/(12*NN)) ; logarithm of the gamma function]
LBPDP=LOG(BIO*PD)
LKTR=LOG(KTR)

PIZZA=LBPDP+LKTR-L
;-----END FOR TRANSIT COMPARTMENT MODEL-----

K20=CL/V2

S2=V2
```

```

$DES
TEMPO=T-TDOS
IF(TEMPO.GT.0)THEN
    KTT=KTR*TEMPO
    DADT(1)=EXP(PIZZA+NN*LOG(KTT)-KTT)-KA*A(1) ; TRANSIT ABSORPTION MODEL
ELSE
    DADT(1)=0
ENDIF
DADT(2)=KA*A(1)-K20*A(2) ; CENTRAL COMPARTMENT

$ERROR
IPRED=F
ADD=THETA(6) ; ADDITIVE ERROR
PROP=IPRED*THETA(7) ; PROPORTIONAL ERROR
W = SQRT(ADD*ADD + PROP*PROP)
IRES=DV-IPRED
IWRES=IRES/W
Y = IPRED + W*ERR(1)

$THETA
(0,40.2) ; CL
(0,390) ; volume
(0,1.97) ; ka
(0,2.2) ; MTT
(1,5.09) ; nn
(0,0.0602) ; ADD ERR
(0,0.25) ; PROP ERR

$OMEGA BLOCK(1) 0.0567 ; BSV CL
$OMEGA BLOCK(1) 0.128 ; WSV CL OCC=1
$OMEGA BLOCK(1) SAME ; WSV CL OCC=2
$OMEGA BLOCK(1) 0.195 ; WSV V OCC=1
$OMEGA BLOCK(1) SAME ; WSV V OCC=2
$OMEGA BLOCK(1) 0.465 ; WSV_MTT OCC=1
$OMEGA BLOCK(1) SAME ; WSV_MTT OCC=2

$SIGMA 1 FIX

$ESTIMATION MAXEVAL=9990 SIGDIG=3 PRINT=5 NOABORT MSFO=MSF5b
METHOD=1 INTERACTION

$COV UNCONDITIONAL MATRIX S PRINT E

$TABLE ID TIME TAD DV IPRED RES IRES IWRES OCC NOPRINT ONEHEADER FILE=SDTAB5b
$TABLE ID TIME TAD CL KA V2 F1 MTT ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 NOPRINT
ONEHEADER FILE=PATAB5b
$TABLE ID WT HT MUAC TSF BSF AGE BSA NOPRINT ONEHEADER FILE=COTAB5b
$TABLE ID SEXM HIV NOPRINT ONEHEADER FILE=CATAB5b
$SCATTER DV VS PRED UNIT
$SCATTER DV VS IPRED UNIT

```

### 3.5 Ofloxacin control stream

```
$PROBLEM OFLOXACIN FINAL MODEL
$INPUT ID TIME DV EDV=DROP BQL AMT EMT=DROP EMDV=DROP MDV EVID
SS II WT HT AGE SEX HIV SET OCC BMI LBW CRCLN CRCLJ RAT
$DATA MDRTB2_ZERO_PRE.csv IGNORE=@

$SUBROUTINES ADVAN6 TOL=3
$MODEL      NCOMP=3
            COMP=(DEPOT, DEFDOSE)
            COMP=(CENTRAL, DEFOBS)
            COMP=(PERIPH, NODOSE)

$PK

;-----CALCULATE TIME AFTER DOSE -----
IF(AMT.GT.0)TDOS = TIME
TAD=TIME-TDOS
;----- END TAD CALCULATION -----

CL1=THETA(1)*((WT/70)**0.75) ; NON-GFR CLEARANCE
CL2=THETA(2)*(CRCLJ/68)      ; GFR CLEARANCE CRCLJ IS CRCL USING LBW IN C-G
FORMULA

CL=(CL1+CL2)*EXP(ETA(1))    ; TOTAL CLEARANCE

TVV2=THETA(3)*(LBW/45.76)    ; CENTRAL VOLUME SCALED TO LBW.
V2=TVV2*EXP(ETA(2))

V3=THETA(4)*WT/70            ; PERIPHERAL VOLUME ALLOMETRIC SCALING FOR
TOTAL BODY WEIGHT

Q=THETA(5)*((WT/70)**0.75)   ; INTERCOMPARTMENTAL CLEARANCE ALLOMETRIC
SCALING FOR TOTAL BODY WEIGHT

IF(SET.EQ.0) THEN
TVMTT=THETA(6)               ; CAPE TOWN MTT
ELSE
TVMTT=THETA(7)               ; DURBAN MTT
ENDIF

MTT=TVMTT*EXP(ETA(3))

NN  = THETA(8)                ; NUMBER OF TRANSIT COMPARTMENTS

;-----CODE FOR TRANSIT ABSORPTION COMPARTMENT MODEL-----
--

IF(AMT.GT.0)PODO = AMT
KTR  = (NN+1)/MTT
L = 0.9189385+(NN+.5)*LOG(NN)-NN+LOG(1+1/(12*NN))
X = 1E-6
LPOD=LOG(PODO+X)
LKTR= LOG(KTR+X)
LPKT=LPOD+LKTR - L

KA=KTR
F1  = 0                      ; dose must enter via transit compartments

;-----END FOR TRANSIT COMPARTMENT MODEL-----
```

```

S2=V2

      K23=Q/V2
      K32=Q/V3

$DES
DEL = 1E-6      ; to prevent log of zero

IF(T.GE.TDOS)THEN
  DADT(1)=EXP(LPKT+NN*LOG(KTR*(T-TDOS)+DEL)-KTR*(T-TDOS))-KA*A(1) ; TRANSIT
  ABSORPTION MODEL
ELSE
  DADT(1)=0
ENDIF
DADT(2)=KA*A(1)-CL*A(2)/V2 - K23*A(2)+ K32*A(3) ; CENTRAL COMPARTMENT
DADT(3)=K23*A(2)-K32*A(3) ; PERIPHERAL COMPARTMENT

$ERROR
  IPRED=F
  ADD=THETA(9) ; additive error
  PROP=IPRED*THETA(10) ; PROPORTIONAL ERROR
  W = SQRT(ADD*ADD + PROP*PROP)
  IF(W.LE.0.0001) W=1
  IRES=DV-IPRED
  IWRES=IRES/W
  Y = IPRED + W*ERR(1)

$THETA
(0,4.66) ; NON-GFR CL
(0,3.68) ; GFR CL
(0,52.1) ; CENTRAL VOLUME
(0,40.2) ; PERIPHERAL VOLUME
(0,58.7) ; INTERCOMPARTMENTAL CL
(0,1.76) ; CPT MTT
(0,0.74) ; DURBAN MTT
(1,6.42) ; NN
(0,0.614) ; ADDITIVE ERROR MG/L
(0,0.0962) ; PROPORTIONAL ERROR

$OMEGA BLOCK(2)
0.0681 ; PPV_CL VARIANCE IN CL
0.0446 0.0918 ; PPV_V VARIANCE IN VOLUME
$OMEGA BLOCK(1) 0.292 ; PPV_MTT

$SIGMA 1 FIX

$ESTIMATION MAXEVAL=9990 SIGDIG=3 PRINT=3 NOABORT MSFO=MSF112
METHOD=1 INTERACTION

$COV PRINT E UNCONDITIONAL MATRIX S

$TABLE ID TIME TAD DV IPRED CWRES NPDE IWRES SET CRCLN CRCLJ SEX WT HIV CL V2
V3 MTT Q KA ETA1 ETA2 ETA3 NOAPPEND NOPRINT ONEHEADER FILE=MYTAB112.TAB

```



### 3.6 Time to event MGIT model control stream

```
$SIZES NO=500
$PROBLEM FINAL TIME TO EVENT MODEL
$INPUT ID HIV TT TIME DV MDV EVID AMT SET CAV ; TT IS TIME ON TREATMENT IN WEEKS.
TIME IS MGIT INCUBATION TIME
$DATA MGIT_TOTAL_RAW_FINAL.csv IGNORE=@
$SUBR ADVAN6 TOL=6

$MODEL NCOMP=2
COMP=(MGIT_GROWTH) ; GROWTH OF BACTERIA IN MGIT CULTURE
COMP=(HAZARD) ; HAZARD COMPARTMENT. OXYGEN CONSUMPTION

$PK

;----- DISEASE PROGRESSION MODEL IN PATIENTS -----;

AA=THETA(1) ; FAST KILL INTERCEPT
ALPH=THETA(2) ; RATE CONSTANT FOR FAST KILL.
FASTKILL=AA*EXP(-ALPH*TT) ; PROGRESSION OF FAST KILL BACTERIAL
POPULATION

IF(CAV.EQ.0) INCR=0 ; NO INCREASE IN SLOWLY KILLED BACTERIA FOR
PATIENTS WITH NO CAVITATION
IF(CAV.EQ.1) INCR=THETA(11) ; INCREASE IN SLOWLY KILLED BACTERIA FOR
PATIENTS WITH NO CAVITATION
IF(CAV.EQ.3) THEN ; FOR PATIENTS WITH NO CAVITATION DATA
  IF(MIXNUM.EQ.1) THEN
    INCR=0 ; FOR MIXTURE OF PATIENTS WITH NO CAVITATION
  ELSE
    INCR=THETA(11) ; FOR MIXTURE OF PATIENTS WITH CAVITATION
  ENDIF
ENDIF

TVBB=THETA(3)*(1+INCR) ; SLOW KILL INTERCEPT. DORMANT/PERSISTENT
MYCOBACTERIA
BETS=THETA(4) ; RATE CONSTANT FOR SLOW KILL. MAYBE RIFAMPICIN
AND PYRAZINAMIDE ON DORMANT MYCOBACTERIA
SLOWKILL=TVBB*EXP(-BETS*TT) ; PROGRESSION OF SLOW KILL BACTERIAL
POPULATION

N0=FASTKILL + SLOWKILL ; TOTAL NUMBER OF MYCOBACTERIA IN PATIENTS
SPUTUM INOCULATION INTO MGIT EACH WEEK

;----- END OF DISEASE PROGRESSION MODEL IN PATIENTS -----;

F_FAST=FASTKILL/N0 ; FRACTION OF FAST AND INTERMEDIATE ORGANISMS
IN SPUTUM INOCULUM
F_SLOW=SLOWKILL/N0 ; FRACTION OF DORMANT ORGANISMS IN SPUTUM
INOCULUM

;----- GROWTH MODEL OF MYCOBACTERIA IN MGIT TUBE-----;

KSTART=THETA(5) ; BASELINE GROWTH RATE OF MYCOBACTERIA IN MGIT
BEFORE COMMENCEMENT OF TREATMENT
KASYMP=THETA(6) ; GROWTH RATE OF MYCOBACTERIA WHEN TT IS
INFINITY. FIXED TO ZERO NOW
VIABILITY=(THETA(7)*F_FAST+THETA(8)*F_SLOW)*EXP(ETA(1)) ; RATE OF DECLINE OF
VIABILITY/FITNESS OF MYCOBACTERIA DURING DRUG TREATMENT
```

KGROWTH=KASYMP-((KASYMP-KSTART)\*EXP(-VIABILITY\*TT)) ; MAXIMUM GROWTH RATE  
OF MYCOBACTERIA IN MGIT TUBE EACH WEEK

NMAX=THETA(9) ; CARRYING FUNCTION FOR LOGISTIC MODEL. MAXIMUM  
CAPACITY

ALAG1=THETA(10) ; LAG TIME OF MYCOBACTERIA FOR EACH INCUBATION  
F1=N0 ; AMOUNT OF MYCOBACTERIA INOCULATED INTO MGIT TUBE  
EACH WEEK

;----- END OF GROWTH MODEL OF MYCOBACTERIA IN MGIT TUBE-----;

IF(NEWIND.LE.1) SURV=1 ; MAKE SURE SURVIVAL IS ONE AT THE BEGINNING

\$MIX

NSPOP=2 ; CAVITATION MIXTURE.

P(1)=0.188 ; PROPORTION OF PATIENTS WITH NO CAVITATION FIXED  
FROM THOSE WHO DID HAVE DATA

P(2)=1-P(1) ; PROPORTION OF PATIENTS WITH CAVITATION PRESENT

\$DES

DADT(1)=A(1)\*KGROWTH\*(NMAX-A(1)) ; LOGISTIC DIFFERENTIAL EQUATION FOR  
GROWTH OF MYCOBACTERIA IN MGIT

DADT(2)=A(1) ; HAZARD OF POSITIVE CULTURE RESULT.

\$ERROR

HAZINST=A(1) ; INSTANTANEOUS HAZARD OF POSITIVE CULTURE

IF(NEWIND.NE.2) OLDCHZ=0 ; DEFINE OLDCHZ

CHZ =A(2)-OLDCHZ ; CUMULATIVE HAZARD

OLDCHZ=A(2) ; REMEMBER PREVIOUS CUMULATIVE HAZARD

SURV =EXP(-CHZ) ; SURVIVAL

IF(DV.EQ.1) Y=SURV\*HAZINST ; PROBABILITY DENSITY OF POSITIVE CULTURE

IF(DV.EQ.0) Y=SURV ; NEGATIVE SPUTUM. NO EVENT

\$THETA

(0,0.0573) ; THETA(1) FAST KILL INTERCEPT

(0,2.68) ; THETA(2) RATE CONSTANT FOR FAST KILL

(0,0.00141) ; THETA(3) SLOW KILL INTERCEPT.

(0,0.124) ; THETA(4) RATE CONSTANT FOR SLOW KILL

(0,8.31) ; THETA(5) BASELINE GROWTH RATE OF MYCOBACTERIA

0 FIX ; THETA(6) ASYMPTOTIC GROWTH RATE OF MYCOBACTERIA

(0,0.863) ; THETA(7) RATE OF DECLINE OF VIABILITY FAST ORGS

(0,0.283) ; THETA(8) RATE OF DECLINE OF VIABILITY SLOW ORGS

(0,0.158) ; THETA(9) NMAX. LIMIT

(0,4) ; THETA(10) ALAG1

(0,0.729) ; THETA(11) CAVITATION COVARIATE ON BB

\$OMEGA BLOCK(1) 0.32 ; ETA(1) PPV VIABILITY

\$ESTIMATION MAXEVAL=9990 SIGDIG=4 MSFO=MSF100 NOABORT  
METHOD=COND LAPLACE LIKE NUMERICAL PRINT=3

\$TABLE ID Y EVID TIME HIV CAV ETA1 HAZINST TT NOPRINT ONEHEADER FILE =  
MYTAB100.tab

### 3.7 Time to event MGIT model control stream with rifampicin AUC covariate

```
$SIZES NO=500
$PROBLEM RIFAMPICIN AUC ON ALPHA SLOPE FROM RUN100
$INPUT ID HIV TT TIME DV MDV EVID AMT SET CAV ; TT IS TIME ON TREATMENT IN WEEKS.
TIME IS MGIT INCUBATION TIME
PZAAUC PZACMAX SLCO RIFAUC RIFCMAX INHAUC INHCMAX ETHAUC ETHCMAX
$DATA MGIT_PK_PD_SIM_FULL_CLEAN.csv IGNORE=@
$SUBR ADVAN6 TOL=8

$MODEL NCOMP=2
COMP=(MGIT_GROWTH) ; GROWTH OF BACTERIA IN MGIT CULTURE
COMP=(HAZARD) ; HAZARD COMPARTMENT. OXYGEN CONSUMPTION

$PK
;----- DISEASE PROGRESSION MODEL IN PATIENTS -----;

AA=THETA(1) ; FAST KILL INTERCEPT
ALPH=THETA(2)*(1+THETA(12)*(RIFAUC-45)) ; RATE CONSTANT FOR FAST KILL.
FASTKILL=AA*EXP(-ALPH*TT) ; PROGRESSION OF FAST KILL BACTERIAL
POPULATION

IF(CAV.EQ.0) INCR=0 ; NO INCREASE IN SLOWLY KILLED BACTERIA FOR
PATIENTS WITH NO CAVITATION
IF(CAV.EQ.1) INCR=THETA(11) ; INCREASE IN SLOWLY KILLED BACTERIA FOR
PATIENTS WITH NO CAVITATION
IF(CAV.EQ.3) THEN ; FOR PATIENTS WITH NO CAVITATION DATA
  IF(MIXNUM.EQ.1) THEN
    INCR=0 ; FOR MIXTURE OF PATIENTS WITH NO CAVITATION
  ELSE
    INCR=THETA(11) ; FOR MIXTURE OF PATIENTS WITH CAVITATION
  ENDIF
ENDIF

TVBB=THETA(3)*(1+INCR) ; SLOW KILL INTERCEPT. DORMANT/PERSISTER
MYCOBACTERIA
BETS=THETA(4) ; RATE CONSTANT FOR SLOW KILL. MAYBE RIFAMPICIN
AND PYRAZINAMIDE ON DORMANT MYCOBACTERIA
SLOWKILL=TVBB*EXP(-BETS*TT) ; PROGRESSION OF SLOW KILL BACTERIAL
POPULATION

N0=FASTKILL + SLOWKILL ; TOTAL NUMBER OF MYCOBACTERIA IN PATIENTS
SPUTUM INOCULATION INTO MGIT EACH WEEK

;----- END OF DISEASE PROGRESSION MODEL IN PATIENTS -----;

F_FAST=FASTKILL/N0 ; FRACTION OF FAST AND INTERMEDIATE ORGANISMS
IN SPUTUM INOCULUM
F_SLOW=SLOWKILL/N0 ; FRACTION OF DORMANT ORGANISMS IN SPUTUM
INOCULUM

;----- GROWTH MODEL OF MYCOBACTERIA IN MGIT TUBE-----;

KSTART=THETA(5) ; BASELINE GROWTH RATE OF MYCOBACTERIA IN MGIT
BEFORE COMMENCEMENT OF TREATMENT
KASYMP=THETA(6) ; GROWTH RATE OF MYCOBACTERIA WHEN TT IS
INFINITY. FIXED TO ZERO NOW
VIABILITY=(THETA(7)*F_FAST+THETA(8)*F_SLOW)*EXP(ETA(1)) ; RATE OF DECLINE OF
VIABILITY/FITNESS OF MYCOBACTERIA DURING DRUG TREATMENT
```

KGROWTH=KASYMP-((KASYMP-KSTART)\*EXP(-VIABILITY\*TT)) ; MAXIMUM GROWTH RATE  
OF MYCOBACTERIA IN MGIT TUBE EACH WEEK

NMAX=THETA(9) ; CARRYING FUNCTION FOR LOGISTIC MODEL. MAXIMUM  
CAPACITY

ALAG1=THETA(10) ; LAG TIME OF MYCOBACTERIA FOR EACH INCUBATION  
F1=N0 ; AMOUNT OF MYCOBACTERIA INOCULATED INTO MGIT TUBE  
EACH WEEK

;----- END OF GROWTH MODEL OF MYCOBACTERIA IN MGIT TUBE-----;

IF(NEWIND.LE.1) SURV=1 ; MAKE SURE SURVIVAL IS ONE AT THE BEGINNING

\$MIX

NSPOP=2 ; CAVITATION MIXTURE.

P(1)=0.188 ; PROPORTION OF PATIENTS WITH NO CAVITATION FIXED  
FROM THOSE WHO DID HAVE DATA

P(2)=1-P(1) ; PROPORTION OF PATIENTS WITH CAVITATION PRESENT

\$DES

DADT(1)=A(1)\*KGROWTH\*(NMAX-A(1)) ; LOGISTIC DIFFERENTIAL EQUATION FOR  
GROWTH OF MYCOBACTERIA IN MGIT

DADT(2)=A(1) ; HAZARD OF POSITIVE CULTURE RESULT.

\$ERROR

HAZINST=A(1) ; INSTANTANEOUS HAZARD OF POSITIVE CULTURE

IF(NEWIND.NE.2) OLDCHZ=0 ; DEFINE OLDCHZ

CHZ =A(2)-OLDCHZ ; CUMULATIVE HAZARD

OLDCHZ=A(2) ; REMEMBER PREVIOUS CUMULATIVE HAZARD

SURV =EXP(-CHZ) ; SURVIVAL

IF(DV.EQ.1) Y=SURV\*HAZINST ; PROBABILITY DENSITY OF POSITIVE CULTURE

IF(DV.EQ.0) Y=SURV ; NEGATIVE SPUTUM. NO EVENT

\$THETA

(0,0.043) ; THETA(1) FAST KILL INTERCEPT

(0,3.89) ; THETA(2) RATE CONSTANT FOR FAST KILL

(0,0.00123) ; THETA(3) SLOW KILL INTERCEPT.

(0,0.108) ; THETA(4) RATE CONSTANT FOR SLOW KILL

(0,7.56) ; THETA(5) BASELINE GROWTH RATE OF MYCOBACTERIA

0 FIX ; THETA(6) ASYMPTOTIC GROWTH RATE OF MYCOBACTERIA

(0,1.1) ; THETA(7) RATE OF DECLINE OF VIABILITY FAST ORGS

(0,0.308) ; THETA(8) RATE OF DECLINE OF VIABILITY SLOW ORGS

(0,0.18) ; THETA(9) NMAX. LIMIT

(0,4) ; THETA(10) ALAG1

(0,0.745) ; THETA(11) CAVITATION COVARIATE ON BB

(0,0.0183,0.0364) ; THETA(12) RIF ON FAST KILL

\$OMEGA BLOCK(1) 0.26 ; ETA(1) PPV VIABILITY

\$ESTIMATION MAXEVAL=9990 SIGDIG=4 MSFO=MSF2012a NOABORT

METHOD=COND LAPLACE LIKE PRINT=3 SIGL=12

\$TABLE ID Y EVID TIME HIV CAV ETA1 HAZINST TT FIRSTONLY NOPRINT ONEHEADER FILE =  
MYTAB2012a.tab

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-----END OF THESIS-----

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